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Effect of anti-microbial extract on physico-chemical changes of selected hydro cooled horticulture produce

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Abstract

The physico-chemical changes of mangoes, bananas, tomatoes and capsicum were measured after being hydro cooled at 12ºC (cooling time of TAT1/2) and stored at refrigerated and ambient temperature for 4 weeks. The results showed that selected hydro cooled fruits and vegetables along with neem extract were significantly better in quality than control. Hydro cooled fruits and vegetables using neem extract showed lower incident of spoilage and less weight loss. Soluble solids concentration, titratable acidity and pH value of fruit were maintained. In conclusion, this study showed that precooling (by hydro cooling) with neem extract and refrigerated storage temperature could maintain quality and extend the shelf life of selected produce.

Keywords: precooling, hydro cooling, cooling time, neem extract, physico-chemical changes, shelf life

1. Introduction

Perishable fruits and vegetables should be cooled immediately after harvest if they are to be shipped or held for extended periods before processing. Cooling removes field heat and reduces microbial activity, thereby preserving quality. Hydro cooling is a common method of rapidly bringing fruits and vegetables to recommended storage temperature. Hydro cooling uses chilled water to cool perishable commodities. Hydro cooling is relatively inexpensive and convenient compared to air cooling, because water is more efficient than air in transferring heat.

Tsang and Furutani, 2006 concluded that water has high thermal conductivity, and uniform contact between cold water and the surface of a product causes temperatures to drop quickly. (Henry and Bennett, 1973) stated that another benefit of Hydro cooling is that it does not dehydrate the products; on the contrary, it may even increase the water content of fruit and vegetable products [1, 2, 3].

Mangoes are a popular, nutritional tropical fruit, which are now one of the most important fruits crops in tropical and subtropical areas of the world. Practical problems relating to mango during post-harvest are overall the shelf-life is short, Post-harvest, mangoes ripen rapidly. Mango is sensitive to storage temperatures below 12ºC, Anthracnose (Colletotrichum gloeosporioides) is the most important post-harvest disease, causing huge losses and wastage of mangoes. Mango being a highly perishable fruit possesses a very short shelf life and reach to respiration peak of ripening process on 3rd or 4th day after harvesting at ambient temperature (Narayana *et al*., 1996) [4, 5, 6]. Patel (2008) reported that hydro-cooling of Amrapali and Alphonso mangoes at 12ºC reduced the weight loss of fruits and maintained the organoleptic qualities effectively during the storage period.

Banana fruits are highly nutritious and easily digestible than many other fruits (Mohapatra, Mishra, & Sutar, 2010).Banana fruits are highly perishable and affected by different microbial contaminates because ripe bananas are very perishable. Fungal diseases is the main factor responsible for great economic loss of banana fruits [7, 8].

Tomato (Lycopersicon esculentum is one of the most important vegetables in India. Tomato is one of the most important "protective foods" because of its special nutritive value. It is extremely perishable and cannot be preserved in fresh stage. Huge post-harvest losses of the harvested tomatoes occur due to inadequate storage facilities, which brings substantial loss to the growers and hence to the national economy. (Sugri *et al*., 2013) reported that as high as 10-20% postharvest losses occur due to delays in transport arrangements and long distances to urban markets [9].

Hydro cooling is more beneficial to peppers than to eggplants because the pericarp of peppers is relatively thin and cooling is rapid. However, Hydro cooling may promote decay, particularly if fruit are not blasted with cool air after they leave the hydro cooler (Ryall and Lipton 1979).

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Peppers or capsicum are consumed either at a physiologically mature but unripe stage (green) or at a fully ripe stage (red, or yellow).Recommended storage conditions for sweet peppers or capsicum are 8-9°C and 95-98% RH (Ryall and Lipton 1979). Capsicum are cooled before shipment or storage either by Hydro cooling or by forced air. Capsicum are sensitive to chilling injury below 45˚F. (Ryall and Lipton 1979) stated that

Hydro cooling is more beneficial to capsicum than to eggplants because the pericarp of capsicum is relatively thin and cooling is rapid. Capsicum prepackaged in moisture-retentive films, such as perforated polyethylene, have a storage life (at 7 to 10°C) up to a week longer than non-packaged capsicum [10, 11, 12, 13, 14].

Banana fruits are highly perishable and affected by different microbial contaminates because ripe bananas are very perishable. One of the most important factors causing great economical loss of banana fruits is postharvest fungal diseases caused by lack of proper handling along postharvest chains.

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Azadirachta indica, commonly known as neem, has attracted worldwide prominence in recent years, owing to its wide range of anti-microbial properties. Neem leaves are known for their versatility and amazing benefits. Neem and its extract can work wonders for various health issues.

Antifungal activity: Extracts of neem leaf, neem oil seed kernels are effective against certain fungi including Trichophyton, Epidermophyton, Microsporum Trichosporon, Geotrichum and Candida.

Antibacterial activity

Oil from the leaves, seed and bark possesses a wide spectrum of antibacterial action against Gram-negative and Gram-positive microorganisms, including M. tuberculosis and streptomycin resistant strains. *In vitro*, it inhibits Vibrio cholerae Klebsiella pneumoniae, M. tuberculosis and M. pyogenes. Antimicrobial effects of neem extract have been demonstrated against *Streptococcus mutans* and *S. faecalis* [15, 16].

The main objective of this work was to assess the effect of neem extract on physico - chemical changes of selected hydro cooled fruits and vegetables and microbial analysis.

2. Materials and Methods

2.1 Materials Require

Hydro cooled produce, weighing balance, ph meter, titrator, refractometer, incubator, colony counter

In this study, Hydro cooled produce stored at ambient and refrigerated temperature were assessed for physico – chemical parameters.

2.2 Methods

Physico - chemical and microbial analysis includes physiological weight loss (%), total soluble solids (TSS), titrable acidity (TA), ph and total plate count were assessed at three days interval for hydro cooled produce with neem extract, hydro cooled with no extract and control stored at ambient and refrigerated temperature.

2.2.1 Physiological Weight loss

The fruits were weighed immediately after imposing the treatment which served as the initial fruit weight and were weighed at three days interval to calculate physiological loss in weight by the difference between initial and subsequent weights and it was expressed as percentage (Karki, 2005) [17].

$$\text{PLW (\%)} = \frac{\text{Initial fruit weight} - \text{Fruit weight on the day of observation}}{\text{Initial fruit weight}} \times 100$$

2.2.2 Total Soluble Solids

Total soluble solids (TSS) were determined with the help of a digital refract meter and the values were reported as °bx, corrected to 20 °C with the help of temperature correlation chart (AOAC, 1975) [18].

2.2.3 pH

Fresh samples was cut into small pieces and macerated with blender and was filtered. The filtrate was used for measuring the pH using pH meter, calibrated to the standard pH 4.0 and 7.0 buffer solutions.

2.2.4 Titratable acidity

The titratable acidity of samples was determined as per the procedure described by Ranganna (1986). 10 g of the ground and filtered sample diluted in 90 ml of distilled water. The volume was made up and aliquot was titrated with 0.1N NaOH using 1% phenolphthalein solutions as an indicator [19]. The percent anhydrous citric acid was calculated as under:

$$\text{Titratable Acidity (\% Citric Acid)} = \frac{\text{Titre x Normality of alkali x Volume made-up x Equivalent weight of acid x 100}}{\text{Volume of alkali taken for estimation x Volume of sample taken x 1000}}$$

2.2.5 Total plate count

Samples of known weight from treatments above were washed in sterile distilled water using a shaker, with fruit to distilled water ratio as 1:9. The wash water was then further diluted using peptone water, up to 10^6 and plated in triplicate on total plate count agar prepared as per manufacturer's instructions and incubated at 25 ℃ for 24 hours (Monaghan *et al*., 2009) [19].

3. Results and Discussion

3.1 Physiological weight loss (%) of selected fruits and vegetables at different storage temperatures (RS - refrigerated storage, AS – ambient storage)

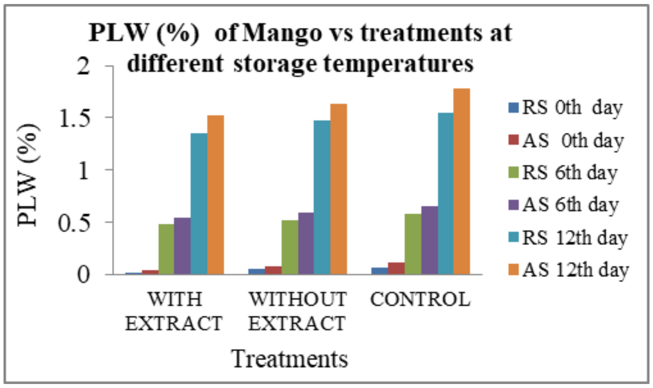

Fig 1

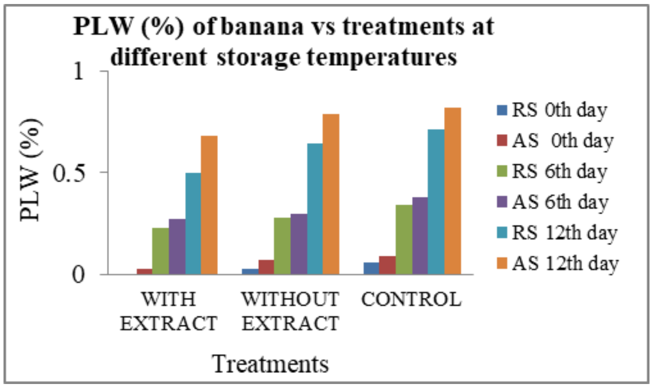

Fig 2

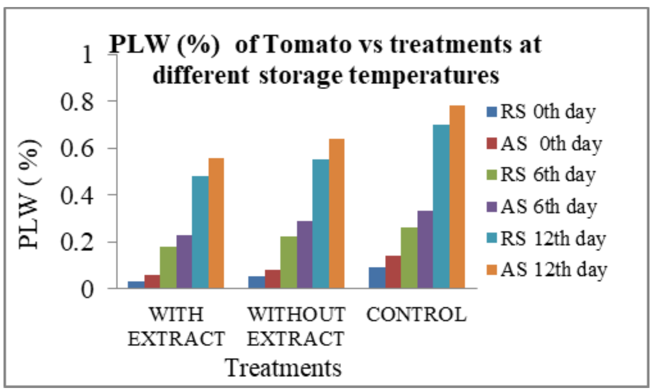

Fig 3

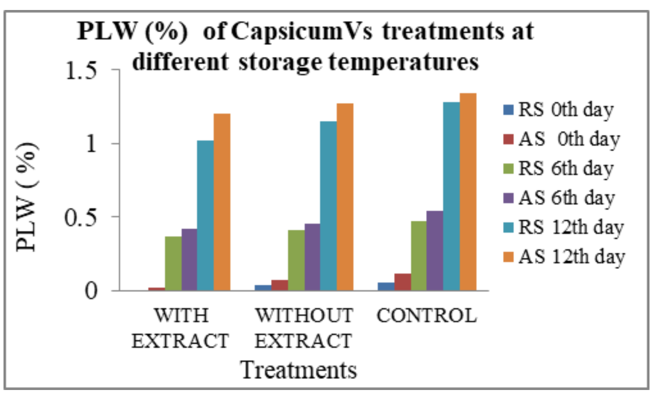

Fig 4

From the above fig 1, fig 2, fig 3, fig 4 concluded that the physiological weight loss (%) in all the produce increased cumulatively and gradually over the storage time regardless of the treatment. The produce hydro cooled with neem extract and kept at refrigerated temperatures showed the least physiological weight loss throughout the storage period reaching 1.35%, 0.50%, 0.48% and 1.02% in mangoes, banana, tomatoes and capsicum respectively. Produce that were not hydro cooled and subjected to ambient storage temperatures and those hydro cooled without neem extract and stored at ambient conditions had the highest physiological weight loss in day 12 as compared to the other treatments. Accordingly, the higher physiological weight loss shown at ambient condition can be associated with increased cell wall degradation leading to exposure of cell water for easy evaporation combined with higher membrane permeability due to faster metabolism and ripening rate at high temperature storage (Dumville and Fry 2000). High temperature increases the vapour pressure difference between the fruit and the surrounding, which is the driving potential for faster moisture transfer from the fruit to the surrounding air (Ryall and Pentzer 1982; Hardenburg *et al*. 1986; Salunkhe *et al*. 1991). In the present study too, the lower temperature and higher relative humidity maintained in refrigerated storage as compared to the ambient condition could be the reason for the low percentage of weight loss possibly through reducing respiration and transpiration rate.

Different produce responded differently to the neem leaves extract. Tomatoes hydro cooled with water containing neem extract showed the least weight loss attaining 0.48 % loss in day 12, remarkably the lowest, for all treatments. The mangoes (1.35 %) and capsicum (1.02 %) had the highest weight loss under the same conditions. i.e hydro cooled produce with neem extract and kept at refrigerated temperature as compared to the other produce. This phenomenon can be attributed to the chilling injuries observed in the mangoes and capsicum under this treatment. This is in agreement with Atta-Aly and Bretch (1995) whose statement revealed that there is significant weight loss as ripening progressed.

Similar findings were also reported by Meseret *et al*. (2012), Tefera *et al*. (2007) and Hiru *et al*. (2008) that weight loss of fruits increased as storage period advanced.

3.2 Total soluble solids

The total soluble solids content in the produce increased throughout the storage period in all produce regardless of the treatments. A significant interaction was observed between the storage time and temperature for all produce. The results of the treatment and storage time and temperature interaction for these produce are as shown below in Fig 5, fig 6, fig 7, fig 8 below.

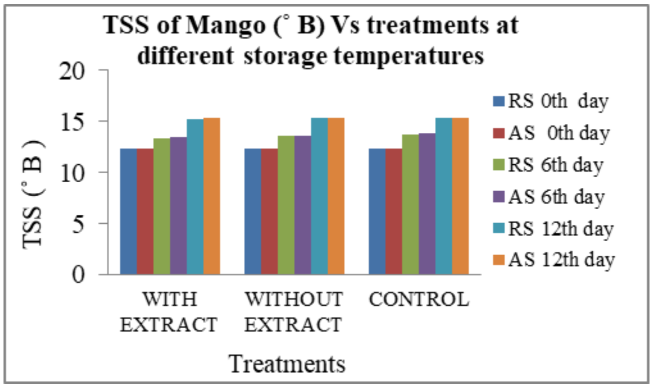

Fig 5

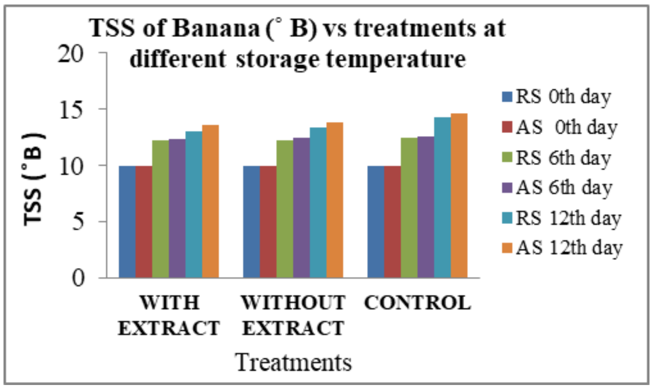

Fig 6

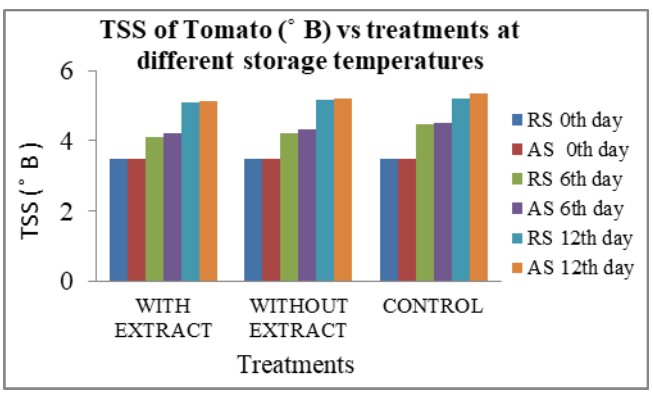

Fig 7

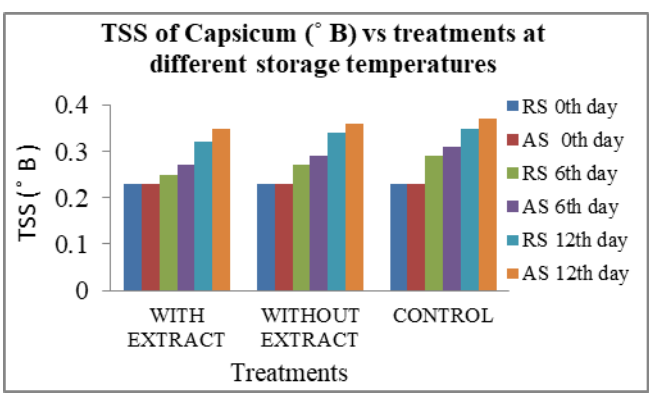

Fig 8

Fig 5, 6, 7 and 8 are mangoes, banana, tomatoes and capsicum respectively, with TSS build-up during storage under various treatments include Produce hydro cooled with neem leaf extract and without neem leaf extract stored at refrigerated and room temperature, non-cooled produce (control).

Neem leaf extract added to the cooling water on the basis surface area of fruit. Hydro cooling had a significant effect in slowing the increase in TSS in all the selected fruits and vegetables. Addition of neem leaf extract to the Hydro cooling water and storage at refrigerated temperature further slowed the increase in TSS.

On day 12, TSS in mangoes and banana in Hydro cooled with extract stored at low temperature had increased from 12.4 (°B) to 15.20 (°B) and 10 (°B) to 13 (°B) compared to the control at ambient temperature which increased to 15.42 (°B) and 14.56 (°B) which was significantly different.

Tomatoes and capsicum showed a similar trend, with TSS in produce hydro cooled with extract and stored at low temperature attaining 5.10 (°B) and 0.32 (°B) on day 12 respectively, which was significantly different from controls at ambient temperature which had 5.34 (°B) and 0.37(°B) respectively.

By the end of the storage period, TSS in neem extract treated samples was lower than the control (water only) in four produce.

(Azene *et al*. (2014); Youssef *et al*. (2012); (Khanbarad *et al*., 2012) reported that the increase in TSS during storage is attributed to the breakdown of starch into sugars or the hydrolysis of cell wall polysaccharides during ripening in tomatoes The lower TSS with Hydro cooling could be due to slowing down of metabolic activities.The slower rate of TSS increase in produce hydro cooled and kept at low temperature is attributed to the effect of precooling which reduced field heat from fruits, restricting respiratory activities and inhibited water loss.

A similar phenomenon was reported by Makwana *et al*. (2014) when mangoes were hydro cooled using water at 8 °C for 2 hours and stored at 8 °C.

3.3 Titratable Acidity

The titratable acidity declined throughout the storage period, in all produce for all treatments as shown in Fig 9, fig 10, fig 11, fig 12 below. Titratable acidity is expressed in the most dominant acid in the specific produce.

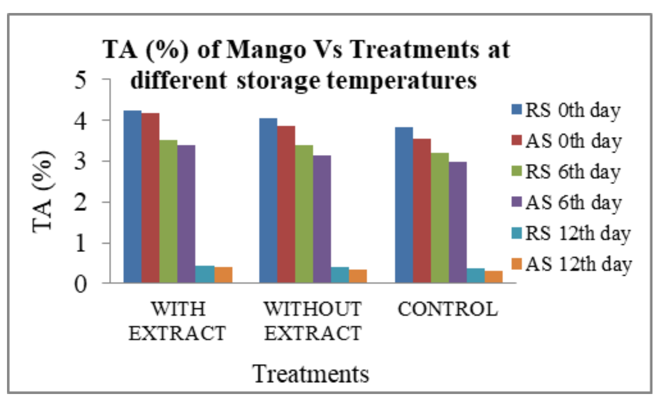

Fig 9

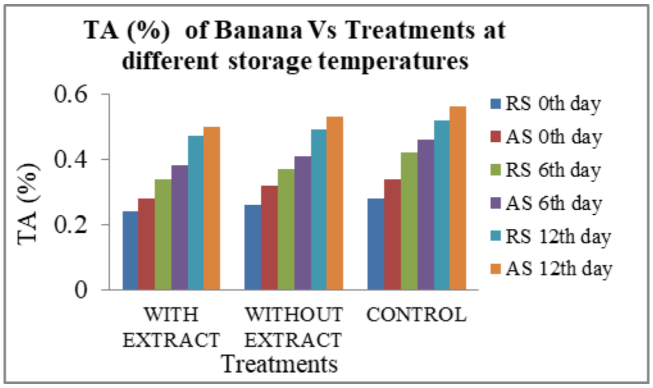

Fig 10

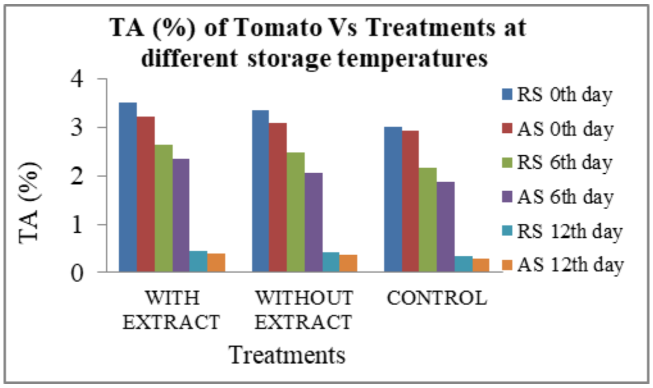

Fig 11

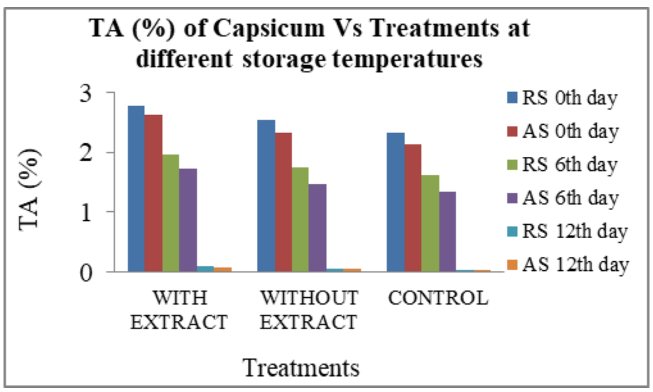

Fig 12

Fig 9, 10, 11 and 12 are mangoes, bananas, tomatoes and capsicum respectively, with Titratable acidity decline during storage under various treatments except, fig 10 banana increase during storage include hydro cooled with extract and hydro cooled without extract, stored at low and ambient temperature and control (not cooled) sample, stored at low and ambient temperature. Neem leaf extract added to the cooling water.

Fig 9, showed that faster decline in acidity was however observed in control stored at room temperature reaching 0.30 % from 4.18% on day 12 in mangoes. The produce that were hydro cooled with extract and without extract, stored at low temperature showed a gradual decline in the acidity attaining 0.43 % and 0.39 % on day 12. This was significantly different from the control.

From Fig 10, banana titratable acidity showed a gradual increase from 0.28% to 0.52%, 0.24% to 0.47% and 0.26 to 0.28% for the control and those hydro cooled with extract and hydro cooled without extract and stored refrigerated temperature and 0.28% to 0.56%, 0.32% to 0.50%, 0.34% to 0.53% for the control and those hydro cooled with extract and hydro cooled without extract and stored at ambient temperature on day 12 respectively.

The initial value of titratable acidity was around 0.24% that increased upto a maximum of 0.56% in control samples after 12th day. The sample hydro cooled with extract stored at refrigerated temperature had lowest acid value after 12th day and it rose further upto 15th day. It confirms the earlier findings by Lustre (1976). Loesecke (1950) reported a similar trend of sharp increase in acidity in course of banana fruit ripening. Values of titratable acidity of the samples, hydro cooled with neem extract, without neem extract and control at both temperature shows that the titratable acid (% malic acid) increased gradually until the fruit reaches to full-ripe stage.

Malic acid has been identified as the main acid in banana, with substantial quantities of oxalic and citric acid in the pulp. While the first two acids are responsible for tartness in the unripe banana, oxalic acid is contributed to astringent taste of the fruit (Seymour, 1993). The malic acid increases upon ripening, whereas the oxalic acid is metabolized and decreases (Salunkhe and Kadam, 1995).

Fig 11 showed that, the tomato acidity declined from 3.02 % to 0.35%, 0.44% and 0.41 % for the control and those hydro cooled with extract and hydro cooled without extract and stored at low temperature respectively.

There was a typical acidity reduction over storage time in tomatoes, associated with ripening. This findings were similar to the results obtained by Shahi *et al.* (2012); Tigist and Workneh (2013) where fully ripe tomatoes had TTA of 0.31% and 0.34 %.Similar findings have been reported by Senevirathna and Daundasekera (2010) and mangoes Rathore *et al.* (2007) or due respiratory activities which utilize organic acids present as substrates (Mahajan & Dhatt, 2004).

(Ţnidarčič and Poţrl 2006) stated that decline in acidity is attributed to ripening, since showed that the amount of organic declined as they are a substrate of respiration in tomatoes. Decline in titratable acidity during storage of perishable produce is related to metabolic processes occurring in them. During respiration, organic acids are utilized as substrates of metabolic processes.

Fig 12 concluded that the titratable acidity in capsicum showed a gradual decline from 2.33% to 0.04%, 0.09% and 0.06% for the control and those hydro cooled with extract and hydro cooled without extract and stored at low temperature and 2.14% to 0.03%, 0.07%, 0.05% for the control and those hydro cooled with extract and hydro cooled without extract and stored at ambient temperature on day 12 respectively.

3.4 pH variations

The pH values in this study seemed to confirm the literature information available on the pH values of pepper (Cochran 1964; Gonzalez-Aguilar *et al.* 1999).

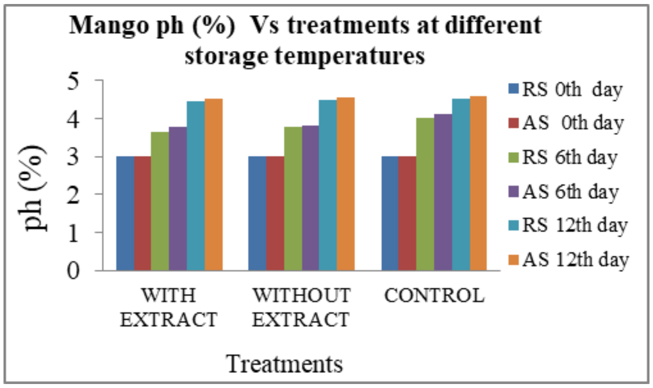

Fig 13

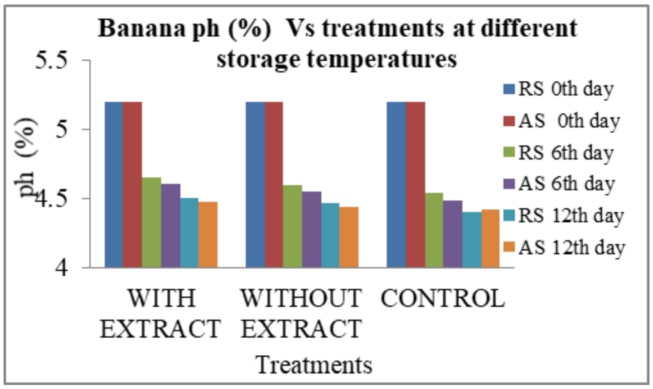

Fig 14

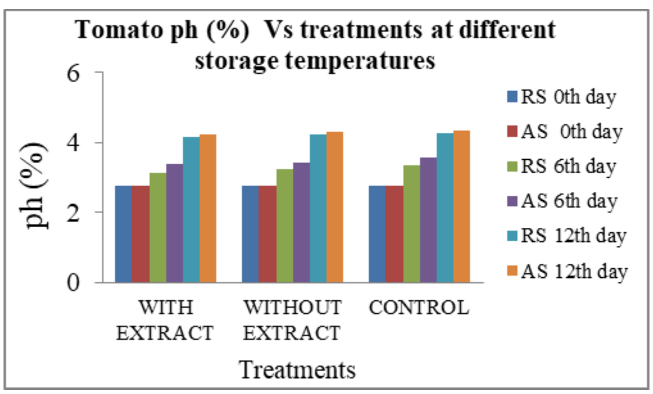

Fig 15

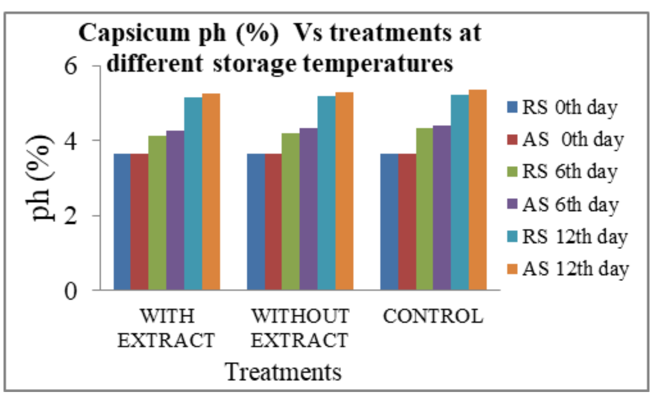

Fig 16

Fig 13, 14, 15, and 16 are ph in mangoes, bananas, tomatoes and capsicum at different storage temperatures respectively, There is a gradual increase in pH in all produce except banana.

From fig 13, Mango showed a gradual increase from 3.02% to 4.53%, 4.45 %, 4.48 % for control, hydro cooled with extract, hydro cooled without extract and control, stored at refrigerated temperature.

Sharp increase in ph from 3.02% to 4.60%, 4.52%, 4.56% was observed for control, hydro cooled with extract, hydro cooled without extract and control, stored at ambient temperature.

From fig 14, ph in banana showed a gradual decrease in ph from 5.2% to 4.54%, 4.68% and 4.70% for the control and those hydro cooled with extract and hydro cooled without extract and stored at low temperature. Sharp decrease in ph from 5.2% to 4.42%, 4.47%, 4.44% was observed for control, hydro cooled with extract, hydro cooled without extract and control, stored at ambient temperature.

From fig 15, The tomato showed a gradual increase in ph from 2.78% to 4.27%, 4.18% and 4.22% for the control and those hydro cooled with extract and hydro cooled without extract and stored at low temperature. Sharp increase in ph from 2.78% to 4.35%, 4.25%, 4.32% was observed for control, hydro cooled with extract, hydro cooled without extract and control, stored at ambient temperature.

From fig 16, The capsicum showed a gradual increase in ph from 3.65% to 5.21%, 5.14% and 5.18 % for the control and those hydro cooled with extract and hydro cooled without extract and stored at low temperature. Sharp increase in ph from 3.65% to 5.35%, 5.24%, 5.30% was observed for control, hydro cooled with extract, hydro cooled without extract and control, stored at ambient temperature. The present result is in conformity with previous reports that showed soon after harvest, overall acidity increased and then decreased in storage (Castro *et al*. 2002). Medlicott and Thompson (1985) also reported the tendency of increasing pH values and reduced acidity with prolonged storage time since the fruit with proceeding of the ripening process is going to diminish its predominant malic acid. According to Mizrach *et al*. (1997), carbohydrate and acid metabolism are closely connected during postharvest ripening period which would thus raise pH of the produce. Getenit *et al*. (2008) also presented an acidity decrease and a pH increase along with maturity evolution.

3.5 Microbiological assay

The microbial quality of the fresh produce varied significantly between the produce. Mangoes had the highest microbial population in total plate count at the beginning of the experiment, before Hydro cooling. Tomatoes had the least in total plate count. The microbial load was generally affected by precooling treatments, the storage temperature and length of storage time. The microbial populations increased with increase in storage time in all the produce. Produce stored at lower temperatures exhibited lower microbial loads. Hydro cooling of all produce with water alone resulted in reduction of microbial populations by approximately half. Addition of the neem extract to the Hydro cooling water further reduced the microbial load in mangoes, bananas tomatoes and capsicum respectively. Produce with neem extract stored at lower temperatures showed lower microbial loads throughout storage time.

Hydro cooling the produce with portable tap water (free of microbial contamination) significantly reduced the initial microbial load in all produce. Although this was contrary to the findings of Mamun *et al*., (2012) in red amaranth leaves, it was similar to those of Workneh *et al*., (2003) in carrots when he compared the effects of tap water, anolyte water and chlorinated water on microbial load.

4. Conclusion

Results obtained from this study showed that neem extract is able to extend the shelf life and quality of selected fruits and vegetables beyond their known natural limits. Neem extract addition to the Hydro cooling water in this study showed varying responses in the selected fruits and vegetables.

The maximum and minimum physiological loss in weight were seen in all four selected produce stored under ambient temperature storage at the end of shelf life and that of refrigerated storage. The physiological loss in weight was significantly lower in neem extract treated fruits under both storage conditions. Refrigerated storage also significantly reduced the weight loss over room temperature storage condition.

The maximum and minimum total soluble solids were observe in all four selected produce under ambient temperature storage at the end of shelf life and that of refrigerated storage. Significantly lower total soluble solids were recorded in neem extract treated fruits. Refrigerated storage had a slower rate of increase in total soluble solids as compared to ambient temperature storage.

The maximum and minimum ph were observe in all selected produce except banana under ambient temperature storage at the end of shelf life and that of refrigerated storage. There was significant influence on pH of fruits during the storage period was observed and under refrigerated storage the rate of increase in pH was slower than room temperature storage.. (Singh & Sharma, 2007) stated the banana fruit contains high levels of sugars and nutrients element, and their low pH values make them particularly desirable to fungal decayed (Singh & Sharma, 2007).

Titratable acidity was the lowest under room temperature storage for all selected produce except banana, than refrigerated storage at the end of shelf life. Precooled fruits maintained significantly higher titratable acidity over the storage period. Fruits kept under refrigerated storage had a higher titratable acidity as compared to room temperature storage.

From this study, it can be concluded that 12°C Hydro cooling with neem extract stored at refrigerated storage was the best treatment for the precooling of mangoes, banana, tomatoes and capsicum as it gave the greater shelf life, less decay and a lower microbial population in all four produce.

By considering the above effect, Mangoes, banana, tomatoes and capsicum hydro cooled with neem extract stored at refrigerated temperature resulting in best quality characteristics throughout the study period.

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