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Influence of Non-toxic- Pollution Free Calcium Hydroxide on Fruits and Vegetable in Different Storage Conditions

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The Fruits and vegetables are highly perishable and their shelf-life is limited to a few days under ambient and refrigeration condition respectively. After the harvest they have no barrier to product them from water loss due to their high respiration rate. When creating an external coating around the fruits and vegetables cuticle surface by dipping chemical or natural solution it forms a semi permeable membrane that extent shelf life of the products. In the present study naturally available oyster calcium hydroxide was used as an external coating agent. In addition grapes, brinjol and tomato samples were dipped with 2% calcium hydroxide solution and stored it in different storage conditions like ambient, refrigeration and Earthen pot cool chamber (EPCC -2) up to 9 days. During the storage period at every 48 hrs interval different parameters like physiological weight (PLW), bacterial and fungal growth, organoleptic quality were studied and recorded. The results showed that pretreatment with 2% calcium hydroxide significantly decreased the loss of physiological weight (PLW), reduced the number of bacterial and fungal growth and retaining high organoleptic quality like texture, flavor, appearance, firmness, taste and particularly color than untreated samples in all the storage conditions. It was concluded that coatings can protect perishable fresh produce from deterioration by retarding dehydration, suppressing respiration, improving texture quality, helping retain volatile flavor compounds and reducing microbial contamination finally extended shelf-life of all the stored produces compared with untreated samples.

Key words: Storage conditions, Fruits and Vegetables, Earthen pot cool chamber, Calcium hydroxide, PLW.

Production and consumption of fruits and vegetables have been growing for the last fifty years particularly due to its nutritional and health benefits. Fruits and vegetables provide all kinds of nutrients including essential minerals, vitamins and dietary fibers etc. Fruits and vegetables remain as living tissues up until the time they are consumed fresh, cooked for consumption, or processed for preservation[1]. When the water reserves are exhausted, the produce dies then subjected to microbial attack and cause spoilage. This is called post harvest losses which mean any change in the quantity or quality of a product after the harvest.

The Post-harvest losses of fruits and vegetables are experienced all over the world but the extent varies. About 10 to 30% of total world grain production is lost after harvest because of inefficient handling, inadequately implemented post-harvest technologies[2]. So extending of shelf-life and the quality of the produce economically feasible storage procedures is essential to minimize losses and assure sufficient supplies over

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long periods of time beyond harvest. The post-harvest handling covers the time span from product harvesting in the farm field until it reaches the urban consumer through the market. There are number of physical, mechanical and chemical methods have been used for fruits and vegetables storage purposes.

But all these methods needs capital costs, operating costs, scale-up possibilities and depreciation of equipment are much cost[3]. The disadvantages are some chemical preservatives which have subtle, deleterious side effects. Preserved food may not always taste as good as fresh food. For the most part, preserved food beats going without. So in order to avoid above mentioned constrains some traditional processing technologies such as dipping, surface coating or skin pretreatment of fresh fruits and vegetables can be done.

Pretreatment generally improves quality and can make the food safer to eat. The skin coating pretreatment is generally easier to treat intact fruits and vegetables than any other process. Because most fruits and vegetables possess a natural waxy layer on the surface, called cuticle and this waxy layer generally has a low permeability to water vapor.

Currently some toxic chemicals are used for pretreatment purpose. But these chemical involves immersion of the product in alkaline or acid solutions of a specific concentration for a specific amount of time[4] and the fruits and vegetables industry are aware of consumer trends and aims to avoid the use of chemical preservatives[5].Besides, consumers hesitate to buy chemically treated fruits, and it has a profound effect on the later drying process.

So as an alternative naturally available or some traditional preservatives may be effective to retain quality of fruits and vegetables. It has some special features like an antimicrobial effect, inhibiting spoilage or avoiding oxidative processes resulting extend the shelf-life of fruits and vegetables. One more advantage in surface skin coating pretreatment is generally easier to treat intact fruits and vegetables than any other process. Resulting, it is ultimately increasing farm incomes, generating rural employment and foreign exchange.

Besides the cytotoxic impact of calcium hydroxide was studied by various researchers and no toxic impact has been observed. The reported that calcium hydroxide-based sealer was the least toxic sealer amongst the chemicals tested in primary human PDL cultures and V79 cultures.[6] The millipore filter technique on rat fibroblast cells for the purpose of determining biological features of four different channel filling paste as in vitro[7]. The examined cytotoxicity of channel filling materials with milipore filters technique in rat fibroblast cells and in human dental pulp cells[8].To detected cytotoxic impacts of materials used in dentistry in primary chicken embryo, fibroblast, and rat fibroblast cells microscopically and via viable cell count[9].

In the present study calcium hydroxide (also called as slaked lime, hydrated lime, slack lime, or pickling lime) was considered as a pretreatment or surface coating agent for fresh fruits and vegetables. Because calcium hydroxide is a most commonly used sanitizer, non-toxic, cost-effectiveness ratio and also traditionally people used calcium hydroxide for preparation of various ethno botanicals such as chewing tobacco, betel nut or coca leaves. Besides, it is an essential mineral that is found in a variety of forms in nature. Usually lime (calcium hydroxide) or sodium hydroxide is used for pretreatment that may be incorporated in the biomass and need to be removed or recycled[10]. Hence, in the present study toxic free calcium hydroxide was used as a pretreatment agent instead of toxic chemicals.

On the basis of the above mentioned some beneficiary effect, in the present study calcium hydroxide salt solution was used to treated the fruits and vegetable and it was stored in different storage conditions like ambient, refrigerator and Earthen pot cool chamber. Different parameters like physiological weight loss, heterotrophic microbial population and organoleptic qualities have been analyzed and the results were tabulated.

MATERIALS AND METHODS

Earthen pot cool chamber (EPCC -2) - Experimental Design

It is a 20 liters capacity of clay pot made of a porous clay material. It contains two water jackets one behind another encircled the pot system for water storing purpose. The size of each water jacket is 5 and 3.5 cm diameter and each jacket to be found at 1 feet interval. (Fig. 1). To facilitates free air circulation inside the pot system there are some holes (2cm diameter each) at bottom and lid of the pot. Before storing the food samples the whole EPCC -2 was immersed in the water for 30 minutes for getting quick cooling. After that 1 lit of water was poured into the both jackets and allowed the system for 2 to 3 hrs. When pot is fed with water; by evaporative principle method the water evaporates into the air raising its humidity and at the same time reducing the temperature of the air inside the pot where kept the food samples for storing purposes. Before and during the whole study period the relative humidity and temperature inside the pot were calculated as 87- 92% and 4-5°C less than room temperature respectively.

Preparation of Calcium hydroxide

Traditional methods of producing calcium hydroxide include heating or burning pulverized oyster shells was used in the present study. Oyster shells were collected from Tuticorin sea shore, Tamilnadu, India. It was cleaned, dried, mixed with charcoal and fired for calcinising the shells. After cooling the powder was sieved with100 mesh. It was used to surface coating treatment experiment by mixing 2 gm of calcium hydroxide powered with 100 ml of water. This was the stock solution.

Sample collection

For this present study freshly harvested uniformly sized vegetables grapes, tomato and brinjol were procured from farmer's field. The samples were collected in sterile plastic bags to avoid the possible contamination from the hands of the samplers[11]. The freshly collected samples were brought to the laboratory within two hours; they were soaked in water to loosen the adhering soil and other foreign materials which were easily removed during subsequent washing operation. Then all fruits and vegetables were washed with sterile water so as to prolong the shelf-life of the fruits and vegetables[12]. After that 180 number of average size, sound and mature grapes, tomato and brinjol were selected for further study. Out of these first 90 numbers of samples were dipped and treated with 2% calcium hydroxide solution for 3 minutes and air dried. After air drying it was covered with plastic bags with respiratory holes for further storage. The remaining 90 number of samples were not dipped and treated with calcium hydroxide and considered as control. Each treated (30 numbers) and untreated (30 numbers) sample were stored at room temperature, refrigerator and in earthenware storage systems respectively for further study. Among each 30 number of samples the first 10 numbers were used to study PLW and another 10 numbers were stored for evaluating organoleptic quality like skin color appearance, texture, taste and flour study purpose respectively. While remaining 10 numbers were used for analyzing microbial study.

All experiments were carried out in an individual room. Rooms were fumigated to remove any air-borne organisms. The fumigation was done by placing 4 gram of potassium permanganate in a Petri dish and adding 1ml of formaldehyde solution to make the fumigation more effective. All the doors and windows were kept closed for 24 hrs before the start of the experiment. All stored vegetables were subjected to study on percentage of physiological loss in weight (PLW), total heterotrophic bacterial and fungal load and percentage changes in organoleptic values and results were recorded for every 48 hrs up to nine days.

Physiological Loss of Weight (PLW)

For PLW analyzing, the initial weight of the each treated and untreated (control) samples were noted and then stored in ambient, refrigerator and EPCC -2 storages. At every 48 hrs interval weight of the each sample were noted and calculated the percentage of PLW for all types of stored samples. The PLW of vegetable samples were calculated by considering the differences between initial and final weight of currently tested vegetables divided by their initial weight[13].

PLW=Initial weight- Final weight X100/ Initial weight

Then the calculated PLW was expressed as a cumulative percentage loss.[14]

Total heterotrophic bacterial count

Before storage, one gram of treated and control samples were cut, homogenized and then serially diluted with saline water. After, serially dilution 'pour plate technique' was performed using plate count agar for bacterial count and PDA for fungal counting respectively. All plates were incubated for 48 hrs at 37°C and 18-22°C for counting bacterial and fungal colonies respectively. Only those plates had isolated colonies of 20–300 were considered. The results were expressed in CFU/gram.[15]By using the same procedure every 48hrs interval the heterotrophic microbial population was calculated and recorded for each sample up to 9 days in different storage conditions. During the study period, the once used samples were not put into the storage again.

Organoleptic quality analysis

Organoleptic quality includes the typical sensory properties of the vegetables: its skin color appearance, flavor, texture, taste and odor were also assessed by using evaluation scale[18]. Samples stored in the three storage systems were presented to a laboratory panel of five judges for sensory evaluation by using suitable method.[16] The panel was asked to rate the samples for skin color appearance, flavor texture, taste and odor on a 10 points hedonic scale using a numerical scale ranging from 2 to 10, where 2,4,6,8 and 10, represented as Poor, fair, good, attractive and very attractive respectively. All the scales were converted into percentage wise. Vegetables scoring lower than 4 out of 10 were considered unacceptable. There were five samples were assessed in each group in each storage in order to get average results. The same vegetable used for analyzing taste and smell was examined only once not put the storage again. All experiments were performed in three replicates in order to assure better analysis.

RESULTS AND DISCUSSION

In the present study the following parameters were analyzed and the results were recorded for both treated and untreated different food samples in different storage conditions.

Physiological loss of weight in treated samples

In the present study the physiological loss of weight (PLW) was calculated at every 48 hrs with initial weigh in all treated and untreated grapes, tomatoes and brinjals samples up to nine days and the results were tabulated in the Figure-2. After the 9 days of storage period the percentage of PLW loss in all stored samples in ambient condition were noted as 16.71% and 9.66%, 08.81% and 08.35% and 13.50% and 11.15% of respectively both in control and treated samples. The percentage differences of PLW in both treated and control grapes samples in ambient storages were noted as 07.05, 00.66 and 2.35. So the treated samples stored in ambient conditions had less percentage of loss of weight than untreated

Table 1. Total viable count of bacteria (10^{-3} CFU) on Grapes stored in different storage systems

S. No	Storage system	Treatment	Storage days 1	3	5	7	9
1	Ambient condition	Untreated	12	45	68	158	TNTC
		Treated	5	12	40	75	87
2	Refrigeration	Untreated	15	25	44	89	127
		Treated	5	9	12	20	34
3	EPCC -2	Untreated	15	22	155	240	TNTC
		Treated	7	12	23	42	67

Table 2. Total viable count of Fungi (10^{-2} CFU) on Grapes stored in different storage systems

S. No	Storage system	Treatment	Storage days 1	3	5	7	9
1	Ambient condition	Untreated	4	17	24	33	47
		Treated	2	5	11	19	21
2	Refrigeration	Untreated	2	5	10	18	37
		Treated	0	2	8	12	16
3	EPCC -2	Untreated	3	6	10	25	72
		Treated	1	3	15	18	32

samples. Like ambient storage all treated samples stored in refrigerator and EPCC-2 showed a less loss in PLW when it compared to untreated samples (Fig. 2). Out of three storages of different samples EPCC-2 had showed low percentage of PLW followed by refrigerator and ambient storage conditions. This might be the calcium hydroxide influence on fruits and vegetables and minimum mechanical and environmental stress, high relative humidity, reduced temperature and minimum evaporation process prevailing inside the cool chamber as compared to room temperature and refrigerator. Stated that stress leads to loss of membrane integrality, leakage, loss of permutation changes in the enzyme activity[11].The reported that the lower temperature and high relative humidity must have reduced the respirational and transpirational losses thereby lower mean weight loss in stored products stored in Zero energy cool chamber (ZECC) [17] and also, reported that vegetables stored in Zero energy cool chamber showed low level of PLW than room and refrigerator storages[14, 18].

In addition to that calcium coating helped reduced loss of PLW in all stored products. Because calcium is an important minerals constituent present in the middle lamellae of fruits and vegetables. Also, the softening of fruits is mainly due to weakening of middle lamellae during ripening. The stated that calcium helps to bind polygalactonic acid and make the membrane strong and rigid[19]. At present calcium treatment has been commercially applied in apple to increase the shelf-life and reduced the post harvest disorders[20]. Therefore, it was concluded that the calcium pretreatment and all favorable conditions in EPCC-2 significantly influenced the shelf-life of stored fruits and vegetables effectively than other two storages.

Total viable count of bacteria and fungi

The total bacterial and fungal populations were significantly high at the end of 9 days of storage in all untreated samples than treated samples. The total viable count (TVC) of bacteria in treated and untreated grape samples stored in different storage conditions were analyzed at every 48 hrs interval up to nine days and the results were noted (Table 1,3 and 5). After nine days of storage in control and treated sample the bacterial numbers were noted as TNTC and 87×10^{-2} in ambient condition, 127×10^{-2} and 34×10^{-2} in refrigerator and TNTC and 67×10^{-2} respectively.

Table 3. Total viable count of bacteria (10^{-2}) CFU on Tomato stored in different storage systems

S. No	Storage system	Treatment	Storage days 1	3	5	7	9
1	Ambient condition	Untreated	15	54	105	225	TNTC
		Treated	12	32	83	107	182
2	Refrigeration	Untreated	20	24	50	98	102
		Treated	4	9	23	52	81
3	EPCC -2	Untreated	14	57	87	190	234
		Treated	8	21	62	93	123

Table 4. Total viable count of Fungi (10^{-2} CFU) on Tomato stored in different storage systems

S. No	Storage system	Treatment	Storage days 1	3	5	7	9
1	Ambient condition	Untreated	10	19	55	62	119
		Treated	2	15	32	38	49
2	Refrigeration	Untreated	8	17	30	43	52
		Treated	7	16	28	47	56
3	EPCC -2	Untreated	5	12	50	61	82
		Treated	1	7	23	37	67

Generally low number of fungi cells were noted for all three treated and untreated samples stored in different storage (Table- 2, 4 and 6). In grape samples there were 47×10^{-2} and 21×10^{-2} colonies in ambient condition, 37×10^{-2} and 16×10^{-2} colonies in refrigerator storage and 72×10^{-2} and 32×10^{-2} colonies in EPCC-2 noted both in treated and untreated samples (Table 2). The same type of results were noted both in treated and untreated tomato and brinjol samples stored in refrigerator and EPCC-2 storages correspondingly. The result analysis showed that the treated samples with calcium hydroxide reduced the microbial density to a greater extent than untreated samples in all storages. All control samples stored in ambient storage had high microbial population compared with refrigerator and EPCC-2 storage. This might be ambient condition is a good medium for the dispersion of microorganisms whereas in refrigerator and traditional earthen pot cool chamber the low temperature did not support the growth of microbes[18]. But all treated samples stored in ambient condition had low microbial number; this might be the activity of calcium hydroxide.

Generally the reduction of microbial load in all treated samples might be due to antimicrobial property of calcium hydroxide and environment conditions in which the food samples were stored. The alkalinization provided by calcium hydroxide damages structural components of the cellular membrane[21], structure of proteins and biological activity of the enzyme and disruption of the cellular metabolism[22] and the Hydroxyl ions of calcium hydtroxide react with the bacterial DNA and induce the splitting of the strands and genes are then lost.[23] Resulting Most of the endodontopathogens are unable to survive in the highly alkaline environment provided by calcium hydroxide.[24] The pre-treatment of fresh produce by calcium hydroxide decreases the density of microbial contaminant from the surface of the fresh produce, reduce the incidence of physiological disorders, storage rots and significantly influenced the extend of shelf-life of tomato fruits. The discovered that calcium hydroxide is effective in reducing brown rot incidence, caused by *M. fructicola,* on unwounded peach fruit[25] and also reported that the maximum shelf-life was noticed in 1% calcium chloride treated fruits (16, 50 days) followed by 0.75% calcium chloride treated fruits (16, 17 days).[19]

Effect of organoleptic quality assessment

After 9 days of storage the organoleptic

Table 5. Total viable count of bacteria (10^{-2}) CFU on brinjol stored in different storage systems

S. No	Storage system	Treatment	Storage days 1	3	5	7	9
1	Ambient condition	Untreated	30	60	158	TNTC	TNTC
		Treated	12	22	58	102	134
2	Refrigeration	Untreated	28	41	109	139	172
		Treated	5	15	60	75	104
3	EPCC -2	Untreated	24	72	115	209	289
		Treated	23	45	59	63	97

Table 6. Total viable count of Fungi (10^{-2} CFU) on Brinjol stored in different storage systems

S. No	Storage system	Treatment	Storage days 1	3	5	7	9
1	Ambient condition	Untreated	6	10	40	130	173
		Treated	2	7	12	27	33
2	Refrigeration	Untreated	5	7	42	78	98
		Treated	3	5	32	38	68
3	EPCC -2	Untreated	5	18	55	96	156
		Treated	3	7	20	32	41

quality of both treated and untreated grape samples were scored as 50 and 58% in ambient, 63 and 74 % in refrigerator and 78 and 81% in EPCC-2 storage conditions respectively (Fig. 3). Generally, the organoleptic percentage differences in treated samples in all storage conditions significantly improved than untreated samples. The same manners of results were obtained for treated and untreated tomato and brinjal samples kept under all three storage conditions. From the study it is clear that the treated samples had maintained better organoleptic quality than untreated samples in all three storage system. Especially, high percentage of organoleptic quality of maintained samples were kept under EPCC-2 than ambient and refrigerator conditions. The reason might very favorable storage conditions (25-27°C and 87-92% of humidity) maintained by EPCC-2 which arrest microbial growth and respiration process and these factors preserve fruits and vegetables for long time, in addition to that the effect of calcium hydroxide on fruits and vegetables appears skin surface. Because calcium plays an important role in fruit physiology as it stabilizes cell membranes and turgor pressure. It interacts with pectic acid in the cell walls of fruit and forms calcium pectate which stabilizes cell structure. It also maintains appearance of fruits and vegetables by inhibiting browning as it reduces leakage of polyphenol oxidase (PPO) and its substrates on cut and exposed surfaces.[26]

The Calcium content of the calcium hydroxide helps to maintain the vegetable cell wall integrity by interacting with pectin to form calcium pectate and generally remain firmer than controls during storage[27]and reducing chlorophyll and protein loss and inhibiting plant tissue senescence.[28],[29],[30] The acknowledged that calcium is reported to maintain firmness by cross-linking with cell wall and middle lamella pectins[31]. In apples it has been reported to reduce respiration and increase firmness retention as well as reducing in general the incidence of physiological disorder and decay[32, 33, 34].

The cytotoxic impact of calcium hydtroxide has been studied by various researchers and observed no toxic impact and reported that calcium hydroxide-based sealer was the least toxic sealer amongst the chemicals tested in primary human PDL cultures and V79 cultures.[6] The milipore filter technique used on rat fibroblast cells for the purpose of determining biological features of four different channel filling paste as in vitro[7.] The examined cytotoxicity of channel filling

Fig. 1. Earthen Pot Cool Chamber (EPCC -2)

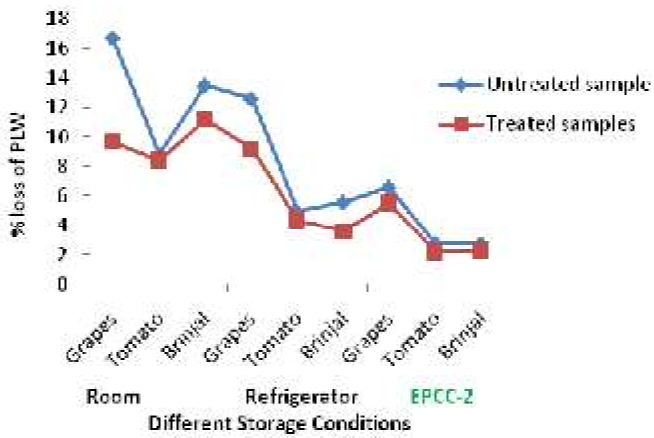

Fig. 2. Physiological loss of weight (in percentage) in untreated and pretreated samples stored in different storage system after nine days of storage.

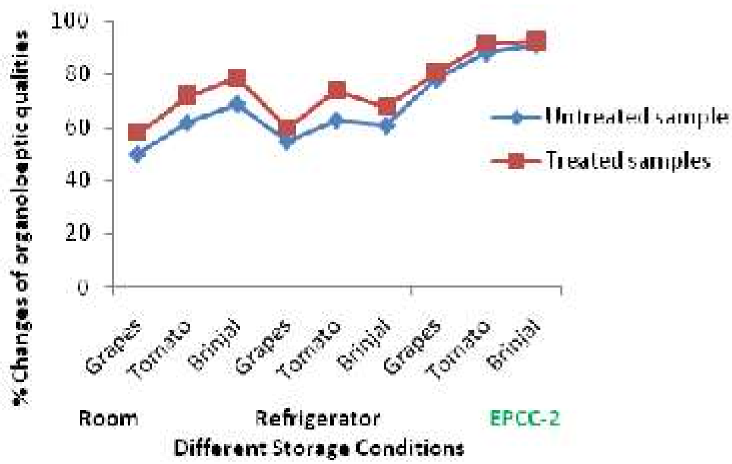

Fig. 3. The percentage changes of organoleptic qualities in control and calcium hydroxide pretreated samples in different storage system after nine days of storage.

materials with milipore filters technique in rat fibroblast cells and in human dental pulp cells[8] and detected cytotoxic impacts of materials used in dentistry in primary chicken embryo, fibroblast, and rat fibroblast cells microscopically via viable cell count[9].

CONCLUSION

The fruit and vegetables market have grown rapidly in recent years due to the health benefits associated with these foods but most of the parts in the world fruit and vegetables storage is very difficult due to lack of infrastructure. Simultaneously the quality of fruit and vegetables products determines the value to the consumer. The consumer's judges evaluate the quality of fruit and vegetables on the basis of physical appearance (organoleptic quality, loss of weight) and microbial spoilage at the time of purchase. So in order to attain this quality in the present study 2.0 % of non toxic, cost-effectiveness calcium hydroxide was used as a pretreatment agent instead of chemical treatment. The treated and untreated samples were kept in three different storages like ambient, refrigerator and EPCC-2 conditions up to

9 days. The result showed that the treated samples stored in EPCC-2 maintained high percentage of organoleptic quality and less microbial hit than untreated samples among the three storage conditions. Also various researchers reported that no cytotoxic impact of calcium hydroxide. So it was concluded that calcium hydroxide can be used an alternative method for chemical treatment and the treated fruits and vegetable kept inside EPCC-2 extending the shelf-life effectively than those were kept in ambient and refrigerator storage. Also, it can be suggested that this treatment was found to be the scientifically feasible and economically viable method for the preservation and storage of fruits and vegetables.

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