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Studies on Quality Changes of Pepino (*Solanum muricatum*) Fruit during Storage

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Abstract:
Physico-chemical characterization of ripe and unripe pepino (Solanum muricatum) fruits was carried out and studied for their quality changes during storage (25 ±8 º C and 7 ± 2ºC). The parameters included physiological loss in weight (PLW %), colour, texture, sugars, ascorbic acid, total phenolic content and antioxidant activity. Pepino packed in polyethylene bags and stored under cold storage were found to be having a longer shelf life. The storage studies were carried out for a period of 28 days and no significant (P < 0.05) differences in moisture, TSS, acidity, pH and MI were observed. Sucrose in ripe pepino (7.19 %) was the predominant sugar followed by glucose and fructose (3.63%). Ripe pepino had higher vitamin C (43.16 mg /100g), total phenolic content (93.02 mg GAE/100g) in comparison to unripe pepino (36.02mg / 100g and 4.16 mg / 100 g GAE respectively). However, the antioxidant activity of unripe pepino was higher (13.52 % ascorbic acid equivalent) as compared to ripe pepino fruits.

Keywords: *Pepino, Shelf life, Storage, Colour, Quality evaluation, Total phenolic content, Antioxidant activity*

1. Introduction

Pepino (*Solanum muricatum*) is an herbaceous perennial member of the solanaceae family. It is grown in Northern Andes region of South America at an altitude of 2700 MSL while in India, it is grown in the Nilgiri districtlies at an altitude of 2500 above MSL (Ahumada and Cantwell, 1996; http://www.tnau.ac.in/dr/zonepdf/HighAltitude HillyZone.pdf). It is cultivated in Peru, Eucador, Chile, Bolivia, New Zealand, and Australia and exported to Japan and USA (Redgwell and Turner, 1986). Pepino fruit named as pepino dulce means "sweet cucumber" in Spanish and pepino in English (National Research Council, 1989). The demand for new, exotic, healthy produce is the main reason for the increasing interest in pepino dulce in the Western world. In addition to South America, the plant is being grown commercially in New Zealand, mainly for export to Japan, and markets are being developed in California, Europe, and Israel (Pluda et al., 1993).

Pepino fruits occur in variety of shapes, sizes, colours and quality. Shapes may vary from round to elongate, globose to pointed oval, its size is similar to muskmelon and it weighs around about 100 – 500 g (National Research Council, 1989; Ahumada and Cantwell, 1996). Skin colour of the fruit may be creamy to yellow orange after ripening. Pulp of the fruit may be greenish, yellow, and salmon. It has a cucumber– like scent flavor would develop before ripening. When ripe, it has a mixture of melon and pear aroma, the fruit must have at least 8% of soluble solids to have an acceptable degree of sweetness (Adrian Rodriguez-Burrueoz *et al*., 2011). Quality of pepino fruit may be improved through nutrition, physiology and chemistry of the pepino (Redgwell & Turner, 1986).

Pepino fruit contains around 92 % water, 6-12% soluble solids, is low in calories (250 Kcal/kg), is rich in vitamin B & C (>200mg/kg) and minerals (K > 1000mg/kg) (Diaz, 2006; Pluda *et al*.,1993; Redgwell & Turner, 1986; Sanchez *et al*., 2000). Vitamin C is important for the bioavailability of dietetic non – heme iron. Vitamin C is ideal for a number of metabolic and antioxidant activities. Vitamin C and various bioactive components found in pepino fruit are responsible for its beneficial health effects (Diaz, 2006; Cartron *et al.,* 2001; Giovanelli & Buratti, 2009; Locatelli *et al*., 2009). Adrian RodriguezBurrueoz *et al*. (2011) reported that the medicinal properties of pepino have an important effect on hypotension, diuretic and antitumoral activity.

Pepino is considered as both climateric and non - climateric fruit, it is based on ethylene production and increased rate of respiration (Lizana and levano, 1977; Heyes *et al*., 1994). Respiration rate of pepino is normally falls under low to moderate. Although, many reports are available on the medicinal properties of pepino fruits, not much work to be carried out on its storage properties.

Pepino is considered as both climateric and non - climateric fruit, it is based on ethylene production and increased rate of respiration (Lizana and levano, 1977; Heyes et al., 1994). Pepino fruit is non – climacteric fruit because of its higher sugar content in the later stages of maturation and ripening (Miguel Ahumada and Marita Cantwell, 1996). Respiration rate of pepino is normally falls under low to moderate. In India, pepino was grown in the Nilgiri district lies at an altitude of 2500mabove MSL. The average rainfall in Nilgiris ranges from 950 to 1550mm (http://www.tnau.ac.in/dr/zonepdf/HighAltitude-HillyZone.pdf). Natarajan *et al*. (2000) studied

and evaluated the performance of nine genotypes of pepino (Solanum muricatum) selected from seedling progenies in the Nilgiris (Tamil Nadu, India) in both main and ratoon crops. Plant growth, days to 50% flowering, fruit characteristics and fruit yield differed significantly among the genotypes. Ratoon crops had better growth and flowered earlier, number and yield of fruits/ plant were greater in the ratoon crop whereas the weight and fruit size was higher in main crop. Among the genotypes, SMu-1 was superior to other genotypes and recorded the highest total number of fruits per plant and mean fruit weight. Hence, genotype SMu-1 was selected and used for this present investigation, and to determine the physico-chemical changes during storage.

2. Materials and Methods

2.1. Material

Pepino (*Solanum muricatum*) SMu-1 fruits were procured from Institute of Commercial Horticulture, TNAU, Nilgiris, Tamil Nadu, India. The harvested fruits (unripe and ripe pepino) were collected and separated into two batches of four fruits in each pack and kept at room temperature (25 ± 8º C) and at low temperature (7 ± 2º C). The shelf-life studies and chemical composition were done on each of the samples. Polyethylene bags of 120-gauge thickness were used for packing the fruit samples and kept for storage studies. Ascorbic acid, glucose, fructose, sucrose, 1,1– diphenyl -2-Picrylhydrazyl (DPPH), gallic acid was procured from Sigma chemical Co., USA. acetic acid (HPLC grade), methanol (HPLC grade), acetonitrile (HPLC grade) and Metaphosphoric acid (MPA) were procured from Merck, Germany. Folin – Ciocalteu reagent (AR), sulphuric acid (AR), sodium carbonate (AR) and methanol (AR), Ethylene diamine tetraacetic acid (EDTA) was procured from Sisco Research Laboratories, India.

2.2. Physical Evaluation of pepino

2.2.1. Shelf life of the pepino

Screened pepino were packed in 120-gauge polyethylene bags. The shelf life of the fresh pepino fruits was studied by storing the fruits in two different storage conditions such as room temperature and at low temperature. Initial weight of the pepino fruits was noted (PLW%) at 7 days' intervals during storage and was carried out for each sample at different storage conditions (Vanitha *et al*., 2005). Quality evaluation was carried out by using rating sca1e of 1-5 (Miguel Ahumada and Marita Cantwell, 1996).

2.2.2. Colour

Colour was measured with a hunter- lab colorimeter (Model lab scan XE, Virgina, USA). Colours of the pepino fruit were taken in three different positions on the surface of each fruit, to avoid areas with purple stripes. Colour measurements were expressed in the L*, a*and b* scale. From these values, the Hue angle 90 and chroma values were calculated (Huyskens-keil *et al*., 2006).

2.2.3. Texture

Fruit firmness was measured by Texture analyzer (TADHi, Stable Micro Systems, UK) with a 2 mm diameter probe. The measurements for each fruit were carried out at three different positions. The average values per sample were recorded and statistically analyzed (Huyskens-keil *et al*., 2006).

2.3. Free Sugars by HPLC

Free sugars like glucose, fructose and sucrose were determined using HPLC (Model LC-10A). A supelcosil TM LC- NH2 (25cm x 4.6mm, 5µm) was used. Acetonitrile /water (80:20) was used as the mobile phase with flow rate of 1ml /min. Free sugars were analysed in unripe and ripe pepino and the results was expressed as mg / g FW (Huyskens – keil, *et al*., 2006).

2.4. Vitamin C by HPLC

Fruit pulp (1g FW) was homogenized with 3% MPA, 8% acetic acid, 0.3 N H_2SO_4, 1 mM EDTA and centrifuged at 4000 rpm at 4°C for 10 min. 5 ml of the supernatant was collected and filtered through 0.45µm filter unit. The filtrate (25 µl) was used for analysis of ascorbic acid (AA). HPLC (Shimadzu, 10 AV UV- vis detector) equipped with an RP 18 5u chromatographic column, length 250 mm was used to determine the ascorbic acid in unripe and ripe pepino. The mobile phase (1mM NaH_2PO_4, 1mM EDTA, pH 3) was used, 25µl of the sample was injected and the flow rate was kept at 1 ml min -1. The method for sample preparation was adapted from Oliveira DDS *et al*. (2010) with slight modification in the sample preparation.

2.5. Total phenolic Content and Antioxidant Activity

Sample extractions were done according to Ndhlala *et.al*. (2008). Fresh fruit sample (2 g) were extracted 1with cold 50% aqueous methanol (10 ml) twice. Extracts were combined and centrifuged at 3000 rpm for 10 min. The collected filterate was used for analysis of total phenolic content and antioxidant activity by DPPH method. Total phenolic content (TPC) in the methanol extract was analyzed by Folin ciocalteau method, and results were expressed as mg gallic acid equivalents per 100g fresh weight. DPPH activity of methanolic extract of fruit sample was determined according to the method of Jakobek *et al*. (2009).

2.6. Statistical Analysis

Statistical analyses of the collected data were subjected to ANOVA and t' test. Analysis of variance was used to determine significant differences ($P < 0.05$) in PLW %, decay and discoloration. Student's t' test was used to determine the difference of free sugars,

vitamin C, total phenolic content and antioxidant activity of unripe and ripe pepino. Duncan's Multiple Range Test (DMRT) was used to compare means of ripeness at 5% significance level. All analysis was carried out in triplicate and expressed as mean ± standard deviation.

3. Results and Discussion

3.1. Shelf Life of Pepino during Storage

Pepino fruits were harvested at different maturity stages and were selected according to colour, shape, and size (100-250g fresh weight and 60-90 mm diameter). The fruits were divided into two batches of ten fruits for each set of storage conditions. Polyethylene (120-gauge thickness) bags were used to pack the ripe and unripe pepino for shelf life studies. PLW % of unripe and ripe pepino was studied and the data collected in every week of storage are given in Table 1. The PLW% was found to be higher in the samples stored at room temperature than at low temperature. Initially, the percentage loss was lesser then it increased during storage. Pepino packed in polyethylene bags and stored at low temperature was found to have longer storage life. The PLW % was found to be null in low temperature storage. Heru Prono – widayat *et al.* (2003) reported that stored pepino at 5°C with 95% RH had limited loss of fruit quality attributes and the similar results were observed in the present study. Significant differences ($p < 0.05$) of PLW% was observed between the ripeness of the fruit and storage temperatures. Shelf life of pepino fruits was extended to one week under Room temperature and four weeks at 10° C with 90-95% RH respectively (EI-Zeftwai *et al*., 1988; Dennis *et al*., 1985)**.**

	Storage days					% Loss
Samples	0	7	14	21	28	Mean ± SD
PUR @ 25 ± 8ºC	1.33	0.55	0.14	0.95	1.14	0.82 ±0.47
PUR @ 7 ± 2ºC	0.9	0	0	0	0	0.18 ± 0.40
PR @ 25 ± 8ºC	1.6	1.42	4.16	4.76	2.5	2.88 ± 1.50
PR @ 7 ± 2ºC	0.32	0.66	0.13	0.1	0	0.24 ± 0.26

Table 1: Physiological loss in weight (PLW %) of PUR and PR during storage
PUR – Pepino Unripe; PR – Pepino Ripe

3.2. Quality Evaluation of pepino during Storage

Quality evaluation was recorded based on 5 scales (1- none, 2- slight, 3-moderate, 4-moderately severe, 5-decay). Discolouration and decay were recorded every week of storage as per the method given by Miguel Ahumada and Marita Cantwell (1996). Fig. 1 shows the visual quality of discolouration and decay of unripe and ripe fruits stored at room temperature and low temperature respectively. Fruits stored at room temperature and low temperatures had no change of colour in first two weeks. Decay was slightly increased in the samples stored at room temperature where as the pepino stored at low temperature had lower percentage loss of decay and discoloration. It was found that the storage temperature for pepino fruits at low temperature had better quality and increased shelf life. Similar trend was noticed in pepino fruit stored for four weeks at 0, 2.5, 5 and 7.5ºC, slight discolouration at 5ºC and no discolouration after storage at 7ºC (Miguel Ahumada and Marita Cantwell,1996). Statistically, there was no significant difference of decay and discolouration between storage days and temperatures.

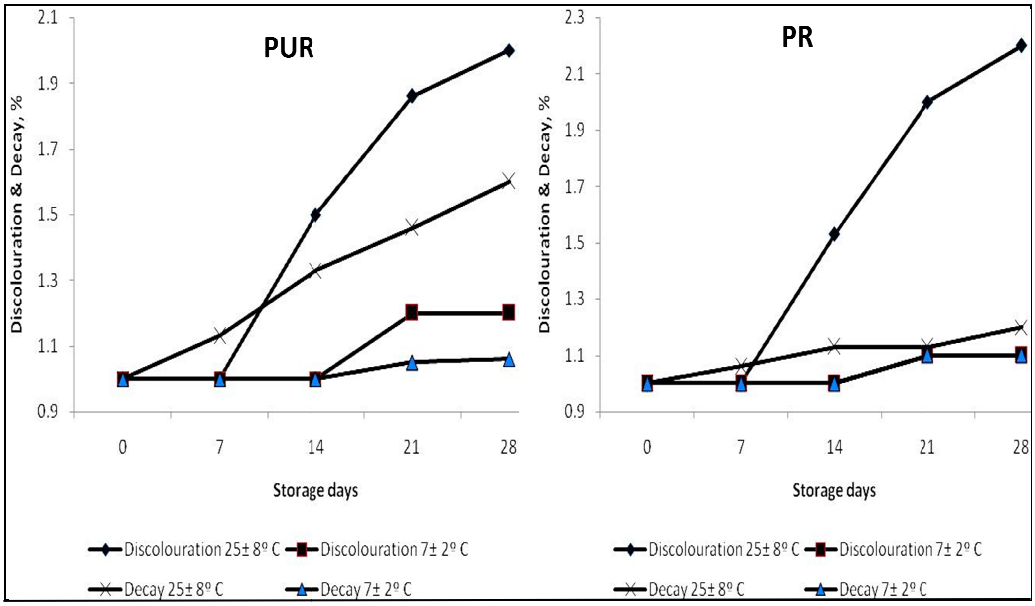

Figure 1: Discolouration and decay of PUR (pepino unripe) and PR (pepino ripe) fruits during storage

3.3. Colour

Colour of pepino changes from green to yellow or orange colour with purple stripes. Completely yellow or orange-yellow colour is demanded for market (Abbott, 1999; Lopez *et al*., 2000). The results revealed that the CIE parameter (L*, a*, b*) decreased and the chroma value increased, during storage periods, as can be observed by the increase in chroma value and decrease in hue angle observed in ripe fruits and vice versa in unripe fruits. Unripe pepino fruits showed a significantly lower chroma than ripe fruits, during the storage periods. A slight change of chroma in ripe pepino occurred in 7th day of storage; it slowly increased after 14th day of storage. Significant differences were noticed in unripe and ripe fruits in room temperature and low temperature, where as there were no significant differences in ripened fruits (0-7 days). The chroma value of pepino fruits significantly increased in ripe pepino, values were highest in fruits stored at room temperature. All values presented in the Table 2, show the mean ± SD of triplicates determination of CIE values and given in different letters are significantly different at $p < 0.05$ by DMRT (Duncan's Multiple Ranging Test). L*, a*, b* values were significantly different in unripe and ripe fruits. The hue angle of the pepino skin decreases consistently with the ripening stage, with value lower than the hue angle and very near to 90º (yellow) (Gonzalez *et al.,* 2000). This report was correlated with findings and similar trend was noticed in this study.

Samples	Storage Days	L*	a*	b*
PUR	0	75.5 ^{b}	1.92 ^{b}	29.15 ^{a}
	7	63.51 ^{a}	5.54 ^{c}	34.1 ^{c}
	14	72.65 ^{b}	2.08 ^{b}	26.45 ^{b}
	21	64.76 ^{a}	0.09 ^{a}	33.62 ^{c}
	28	76.38 ^{c}	1.94 ^{b}	29.55 ^{a}
PR	0	42.55 ^{a}	3.5 ^{a}	13.5 ^{a}
	7	43.25 ^{a}	3.77 ^{a}	14 ^{a}
	14	71.63 ^{c}	1.95 ^{e}	28.61 ^{b}
	21	66.38 ^{b}	1.26 ^{b}	31.79 ^{c}
	28	73.22 ^{c}	3.16 ^{a}	35.47 ^{d}

Table 2: Value of CIE parameters in PUR and PR during storage
Mean scores with different superscript differ significantly by DMRT at P <0.05

3.4. Texture

Fruits may change in texture during maturation and especially during storage, when they may rapidly become softer. Excessive loss of moisture may also affect the texture of crops (Abbott, 1999). In this study, the firmness of pepino fruits measured by Texture analyzer (TAD Hi), probe diameter was 2mm, and penetration of the probe was 100 mm min^{-1}, firmness was measured on whole fruit from opposite sides of each whole fruit. After 0, 7, 14, 21, 28 days, three fruits were taken from each batch for textural analysis. Results of Table 3 revealed that the force (N) required for the unripe pepino fruits was more than the ripened fruits. Results show that there was a sudden increased force on the 7th day of storage and decreased after 14th day of storage. Forces required for fruit penetration were decreased after ripening of the pepino fruit. Mean scores of force (N) were significantly different at $P < 0.05$ by DMRT. Heyes *et. al* (1994) concluded that softening of pepino fruits is reported to be associated with the breakdown of structural cell wall carbohydrates. Textural changes of pepino might be due to changes in pectic substances of pepino fruits during storage which results in weakening of the cell walls and reduction of the cohesive forces binding cells together (Heyes *et al* 1994; O' Donoghue *et al*., 1997). There was no significant difference at the end of storage in ripe pepino fruits.

Storage days	PUR	PR
	Force (N)	Force (N)
0	9.91 ^{c}	7.86 ^{c}
7	12.07 ^{d}	11.907 ^{d}
14	6.338 ^{b}	5.518 ^{a}
21	6.084 ^{b}	6.067 ^{b}
28	5.739 ^{a}	6.062 ^{b}

Table 3: Textural Analysis of Pepino during storage
Mean scores with different superscript differ significantly by DMRT at P <0.05

3.5. Sugars in Pepino Fruits

Free sugars play an important role in flavour characteristics of pepino fruit (Sanchez *et.al*., 2000). The free sugar in fruits determined using HPLC (Table 4) shows that the sugars in unripe stage were glucose (2.70%), sucrose (0.20%), and fructose (1.29%) respectively where as in ripe the sucrose content increased (7.21%). Fructose and glucose in ripe pepino were slightly increased. Sucrose content was detected in smaller amounts (0.20%) in unripe pepino. Calibration curve was linear over the range (0.005-0.020 mg/ml) for glucose (r^2 =0.981), fructose (r^2 =0.967) and sucrose (r^2 =0.939) using sigma standards. Sucrose content increased as fruit ripened, this being significantly different or each group reported by Lopez *et al*. (2000). These findings coincide in part with those of Schaffer *et.al*

(1989) who indicated that pepino could be classified as a sucrose accumulator such as sweet melon, showing a reduction in starch and reducing sugars, and an increase in sucrose during ripening. Similar trend was observed in this study. Redgwell and Turner (1986) stated that the sugars in ripe pepino consisted of sucrose, fructose and glucose with sucrose accounting for 50% of the total. Measurement of sugars in the fruit can provide an indication of the stage of ripeness or maturity of that fruit (Thomson, 2003).

Samples	**Free sugars (g / 100 g FW)**		
	Glucose	**Sucrose**	**Fructose**
PUR	2.70 ± 0.01	0.20 ± 0.005	1.29 ± 0.005
PR	3.61 ± 0.015	7.21 ± 0.03	3.60 ± 0.02
t' value	0.00	2.91*	1.9

Table 4: Free sugars in Unripe and ripe pepino fruit

3.6. Vitamin C

Extraction steps of vitamin C in fruits require more care, to prevent the enzymatic action by reducing the pH, what favors AA stability. Furthermore, the presence of metals such as iron and copper increase AA oxidation and a metal chelating agent is usually recommended. Pure water or acid solutions have been used to extract AA from plant tissues. The acids commonly used for extraction includes MPA and oxalic acid alone or in combinations with other acids or organic solvents, added or not of antioxidants such as EDTA and BHT (Flavia Milagres Campos *et al.*, 2009). The samples were extracted by using 3% MPA, 8% acetic acid, 0.3N sulphuric acid, 1mM EDTA, according to the method proposed by Oliveira DDS *et.al* (2010) with some modifications. Vitamin C in the samples was quantified by the calibration curve. The standard stock solution (1mg / ml AA) was prepared in ultra pure water and solutions of various concentrations were prepared by diluting the stock solution. Calibration curve was linear over the range (0.1- 0.5 mg/ml) for ascorbic acid (r^2 =0.997). In the present study, the vitamin C content in unripe and ripe pepino was 36.02 and 43.16 mg/ 100g respectively. Pepino fruit has high levels of ascorbic acid 48-68 mg/100g fresh fruit (Redgwell and Turner, 1986; Rodriguez-Burruezo *et.al.,* 2004). vitamin C content was higher in pepino than normally found in most fruits, including citrus.

3.7. Total Phenolic Content and Antioxidant Activity of Pepino

Total phenolic content in the pepino fruits studied ranged from high in PR (47-93.02 mg GAE/100g FW) and low in PUR (2.1-4.16 mg GAE/100 FW). The standard gallic acid (µg/µl) was used to calibrate the curve and obtained the linear response (r^2=0.981). The total phenolic content in the high range contained very high levels of antioxidants in comparison with commonly consumed fruits such as citrus fruits (reported contained 31-760 mg GAE/100g FW) (Abeysinghe *et al.,*2007; Sun *et al.,* 2002). Apples, pears and red grapes contain 270, 54 and 182 mg GAE/100g FW of total phenolic compounds, respectively (Sun *et al*., 2002). Antioxidant activity was measured by DPPH scavenging method. DPPH is stable organic nitrogen radical that gives a deep-purple colour in methanol. The colour fades upon reaction with phenolic compounds in the test solution (Huang *et al.,* 2005). Antioxidant activity was higher in unripe than the ripe pepino fruits (Table 5).

S. No.	**Parameters**	**PUR**	**PR**	**t'value**
1	Vitamin C (mg / 100g)	36.02 ± 0.02	43.16 ± 0.05	3.40*
2	Total phenolic content (mg GAE/100 g)	4.16 ± 0.05	93.02 ± 0.02	7.85*
3	Antioxidant activity (% as equivalent to ascorbic acid)	13.52 ± 0.01	6.92 ± 0.02	7.82*

Table 5: vitamin C, total phenolic content and antioxidant activity of pepino fruits

4. Conclusion

Significant changes were observed in texture and colour of ripe and unripe pepino during storage. During storage, the decay and discolouration was very less (1-1.5%) in both ripe and unripe pepino. Storage temperatures (7±2ºC) were suitable for extending the shelf life and quality up to 28 days. Regardless of the storage temperature, unripe and ripe fruits showed insignificant changes in the biochemical characteristics during storage. Vitamin C, total phenolic content and sucrose content were higher in ripe pepino than unripe. Ripe pepino contain high amount of ascorbic acid, and display high antioxidant activity. Further work is required to investigate the isolation and identification of individual phenolic compounds with respect to antioxidant activity.

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