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COMPARATIVE EFFECT OF 1-METHYLCYCLOPROPENE (MCP) AND $KMNO_4$ ON THE TOTAL ANTIOXIDANT CAPACITY, PHENOLS AND FLAVONOIDS OF GUAVA CV. LUCKNOW-49

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ABSTRACT : The 'poor man's apple' guava is a rich source of high-grade antioxidants such as Vitamin C, lycopene, carotenoids, polyphenols and flavonoids compared with other tropical fruits. The content of antioxidants in the fruit varies with the storage period, temperature and any pre-treatment given to it. To study the effect of ethylene action inhibitors and absorbents on the total antioxidant capacity of the guava fruits, they were treated with 1-MCP (Methylcyclopropene) @ 500 ppb and $KMnO_4$ @10 per cent and stored at different temperatures. The total antioxidant capacity and total flavonoids content increased during ripening and was high in 1-MCP treated fruits compared to control while, the total phenolic content decreased during ripening and remained maximum in untreated fruits followed by 1-MCP treated fruits.

Key words : 1-MCP, Guava, $KMnO_4$, antioxidants

Natural antioxidants, particularly in fruits and vegetables, have been of increasing interest to both consumers and scientists, such as epidemiologists, food scientists, chemists, and plant scientists because epidemiological studies have indicated that frequent consumption of natural antioxidants is associated with a lower risk of cardiovascular diseases and cancers (Renaud *et al.*, 1998; Temple, 2000). The defensive effects of the natural antioxidants in fruits and vegetables are related to the three major groups: vitamins, especially vitamin C; phenolics; and carotenoids, especially â-carotene (Klein and Kurilich, 2000). Vitamin C and phenolics are known as hydrophilic antioxidants, while carotenoids are known as lipophilic antioxidants.

Guava fruit (*Psidium guajava* L.) contains a high level of antioxidant compounds, such as vitamin C (50-300 mg/100 g fresh weight, which is higher than that in an orange by three to six times) (Nakasone and Puall, 1998); carotenoids, such as â-carotene and lycopene (Mercadante *et al*, 1999); and phenolic compounds, such as ellagic acid and anthocyanin (Misra and Seshadri, 1968) and flavonoids. The free-radical scavenging capability and consequent antioxidant properties of the phenolics play an important role in protecting the cells and tissues from oxidative stress and other biological effects associated with these chronic diseases. To obtain these powerful benefits of antioxidants in guava, which comes in a variety of shapes (round to oval) and colors (white to red), one should take the fruit when they are about to ripen for polyphenols, ripened for Vitamin C and the yellow to red variety for lycopene and carotenoids. The quantity of these natural antioxidants in guava fruits vary during storage and the present experiment was designed to know the influence of 1-MCP (Methylcyclopropene) application on the total antioxidant capacity, total phenolic and flavonoids content of guava fruits cv. Lucknow-49 stored at different temperatures.

MATERIALS AND METHODS

Physiologically mature green fruits of guava cv. Lucknow-49 were harvested manually from the orchards of IIHR during early hours (8.00-9.00 am). Later the fruits were kept in plastic crates and transported to the laboratory, where they were sorted out to remove immature, misshaped, bruised, diseased and insect infested fruits. These fruits were graded as floaters (=1) and sinkers (>1) based on their specific gravity among which floaters (fully matured) were taken for the experiment. The fruits were then washed, air-dried and subjected to various treatments i.e. control, 500 ppb 1-MCP and 10 % $KMnO_4$ solution impregnated into chalk sticks and dried. To obtain 500 ppb 1-MCP, a calculated amount (18 mg) of amorphous 1-MCP powder was taken in a 15 ml test tube which was sealed hermitically with a rubber septa and 1 ml of distilled water was injected into it using a syringe. By shaking the test tube gently the 1-MCP powder dissolves in distilled water to release gaseous 1-MCP. From this gaseous 1-MCP, 0.6 ml was taken using a calibrated syringe and injected into 18 liter capacity desiccators holding 6 kg of guava fruits each. Finally after 18 hours of exposure, the treated fruits were taken out and packed in non-ventilated CFB boxes having three replicates, each of 3 kg fruits and stored under three different temperatures i.e. ambient condition (22-28°C and 80-85 % RH) and low temperatures 8 and 12°C. In case of $KMnO_4$ treatment, 30 g of impregnated chalks were placed in each box.

Total antioxidants were estimated using FRAP (Ferric Reducing Antioxidant Potential) method as described by Benzie and Strain (1996). FRAP reagent was prepared freshly before use by using Acetate buffer, TPTZ (Tripyridyl triazine) and ferric chloride in 10:1:1 ratio. The methanol extract (0.2 ml) of the sample was taken in test tubes and 1.8 ml of working FRAP reagent was added. Then the tubes were kept for incubation at room temperature for 25-30 minutes. The color so developed was read spectrophotometrically at 593 nm and expressed as ascorbic acid

equivalents. Standard curve was drawn using ascorbic acid as standard. Different concentrations of ascorbic acid were prepared and O.D was read at 593 nm. The concentration of samples was calculated based on the standard curve.

Total phenols were estimated according to procedure given by Singleton and Rossi (1965). A 5 g sample was extracted with 50 ml 80% methanol. The extracts (0.5 ml) were taken in the test tubes and were added with 0.2 ml of Folin and Ciocalteau's Phenol Reagent (1N). To that, 3.25 ml of distilled water was added and all the tubes were shaken well. Then, 1 ml of Sodium Carbonate (20%) solution was added to all the tubes and kept for incubation at room temperature for 30 minutes. The colour so developed was read spectrophotometrically at 700 nm. Standard curve was drawn using gallic acid as standard. Different concentrations of gallic acid were prepared and O.D was read at 700 nm. The concentration of samples was calculated based on the standard curve.

Total flavonoid in the methanol extract was determined as per Chun *et al.* (2003). Methanol extract (1 ml) was taken in the test tubes and was mixed with 0.3 ml of 5% $NaNO_2$. After 2 min, 0.3 ml of 10 % $AlCl_3$ was added to it followed by 3.4 ml of 4 N NaOH after 2 min. The absorbance of the pink mixture was read at 510 nm after 5-10 minutes of incubation at room temperature. Standard curve was drawn using catechin as standard. Different concentrations of catechin were prepared and O.D was read at 510 nm. The concentration of samples was calculated based on the standard curve.

RESULTS AND DISCUSSION

Total antioxidant capacity

The changes in total antioxidant capacity of the guava fruits are shown in the Table 1. The total antioxidant capacity of the guava fruits increased at ripe stage compared to harvest. However, the results obtained were contradictory to Neeraj *et al*. (2002) who reported the decline of antioxidants in fruits during their ripening. Among the storage temperatures, guava fruits stored at 12°C had shown higher amounts of total antioxidant capacity followed by those stored at RT and lowest at 8°C. The reduced antioxidant activity at 8°C might be due to more utilization of the antioxidants to neutralize the free radicals produced by the low temperature stress (chilling injury). Among the pre-treatments highest anti-oxidant capacity was noticed in 1-MCP treated fruits at full ripe stage, while the $KMnO_4$ treated fruits had the lowest antioxidant capacity. The 1-MCP treated guava fruits stored at RT had shown significantly higher total antioxidant capacity than other treatments which shows the loss in antioxidants at 12°C was mainly due to increased storage period at this temperature compared to RT.

Total Phenols

The total phenol content declined during storage from the day of harvest (540.25 mg gallic acid equivalence/ 100 g). This decline in the total phenol content might be due to their utilization in the metabolic pathways for production of volatile aroma cum flavor compounds. Similar decline in phenolic compounds during storage have been reported in fruits like apple (Smock and Neubert, 1950), peach and persimmon (Ben-Aire and Guelfat-Reich, 1976). Total phenol content of guava cv. Sardar fruits started declining from maturation and was lowest on 16th day of storage (Ramachandra *et al*., 1995). Singh (1980) observed that total phenol content decreased in guava fruits, but it was more pronounced at room temperature. Total phenols were high in fruits stored at 12°C, followed by RT whereas the fruits stored at 8°C have shown reduced total phenol content (Table 1). Shukla (1977) reported decline in tannin content in mango cv. Dashehari during storage at room and low

Table 1. Effect of 1-MCP and $KMnO_4$ on total anti-oxidant capacity, total phenols and total flavonoids in guava cv. Lucknow-49 fruits stored at different temperatures

Treatments	Total antioxidant capacity (mg ascorbic acid equivalence/100g) At harvest (422.62)				Total phenols (mg gallic acid equivalence/100g) At harvest (540.25)				Total flavonoids (mg catechin equivalence/100 g) At harvest (54.09)			
	RT (T_1)	12°C(T_2)	8°C(T_3)	Mean C	RT (T_1)	12°C(T_2)	8°C(T_3)	Mean C	RT (T_1)	12°C(T_2)	8°C(T_3)	Mean C
Control (C_1)	564.5	691.2	573.9	609.8	499.4	476.6	386.9	454.3	73.37	82.91	67.36	74.55
$KMnO_4$ (C_2)	488.9	654.4	313.5	485.6	377.7	533.4	274.2	395.0	87.19	81.59	69.10	79.29
1-MCP (C_3)	734.9	673.8	436.0	614.9	512.9	463.3	372.8	449.7	89.29	70.63	81.13	80.35
Mean T	596.1	673.1	441.1		463.3	491.0	344.6		83.28	78.38	72.53	
	T	C	T×C		T	C	T×C		T	C	T×C	
F-Test	**	**	**		**	**	**		**	**	**	
S. Em ±	1.374	1.374	2.380		0.831	0.831	1.440		0.382	0.382	0.662	
C.D. @ 1%	5.528	5.528	9.576		3.344	3.344	5.793		1.435	1.435	2.486	

*Significant @ 5%

Temperatures (T) :
T_1=Room temperature
T_2=Low temperature at 12°C
T_3=Low temperature at 8°C

Pre-treatments (C):
C_1=Control
C_2 = $KMnO_4$
C_3 = 1-MCP

** Significant @ 1%

temperatures. Similar observations were recorded in fruits of date (Maier and Metzler, 1965) and ber (Bal *et al*., 1978; Bal, 1982). Hussain *et al*. (1998) stored guava fruits at 10 or 20°C for 3 weeks and found that total phenols decreased significantly as storage period and temperature increased. Among pre-treatments untreated fruits had highest total phenol content followed by 1-MCP treated fruits whereas $KMnO_4$ treated fruits had the lowest phenol content. This might be due to, the number of days required to reach ripe stage was much less in control fruits.

Total Flavonoids

Flavonoids are one of the major compounds contributing for the total antioxidant capacity of the fruits and vegetables. In nature, very large quantity of flavonoids was in the form of catechins. In the current experiment, there were high total flavonoids at full ripe stage compared to day of harvest and the total flavonoids were highest at room temperature (RT) followed by 12°C and lowest at 8°C (Table 1). The highest flavanoid content at room temperature might be due to lesser number of days taken by them to reach full ripe stage. Among the pre-treatments, 1-MCP treated fruits had higher flavonoids followed by $KMnO_4$ treated fruits and lowest was found in control.

This study helps to know the effect of 1-MCP on guava fruit in extending shelf life and retaining the quality of fruits. The total antioxidant capacity and total flavonoids content increased during ripening and was high in 1-MCP treated fruits compared to control while, the total phenolic content decreased during ripening and remained maximum in untreated fruits followed by 1-MCP treated fruits.

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