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HARVESTING TIMES MODULATES THE HUMAN HEALTH PROMOTOING SUBSTANCES IN GRAPEFRUIT

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Grapefruit is an important fruit crop in the world as well as in Pakistan. Phenolic compounds, antioxidants, carotenoids, flavonoids and limonins are important health promoting substances which are present in grapefruit and are considered as preventative against cronic diseases. Therefore, the objective of this study was to investigate the effects of harvesting times on these substances. Fruits of two grapefruit cultivars i.e. Ray Ruby and Shamber were harvested at different dates viz. 1st September, 1st October, 1st November, 1st December and 1st January during the both study years. The fruit harvested in the month of December showed higher total phenolic compounds (172.25 and 176.62 mg GAE/100g), total antioxidants (72.30 and 79.14 %), total carotenoids (19.34 and 21.87 mg/100 g), total flavonoid contents (69.83 and 69.90 mg CEQ/100g), total limonin contents (10.96 and 10.51 µg/mL), total pectin contents (10.65 and 10.81%), total glycoside limonin contents (194.97 and 204.89 µg/mL) in Ray Ruby and Shamber cultivars of grapefruit, respectively. All other quality components such as TSS, ascorbic acid and sugars were also increased up to December and then started to decline. It is concluded that the Ist December is an optimum time for harvesting of both Ray Ruby and Shamber grapefruit cultivars in relation to health promoting substances.

Keywords: Essential phytochemicals, limonin, antioxidants, harvesting times, ascorbic acid, citrus

INTRODUCTION

Citrus fruit is grown in more than 64 countries of the world (Chaudhry *et al*., 2004) with a total production of 105.4 million tonnes annually (PHDEC, 2006). Citrus rank first with respect to area and production in Pakistan (Anonymous, 2013). Pakistan stands at 13th position among the citrus producing counties of the world with a total area of 199,000 acres and total annual production of 2.36 million tonnes (FAO, 2013). Citrus fruit can be divided into four main groups such as mandarins, sweet oranges, pummelo and grapefruit, and lemon and limes species. Grapefruit is ancestor of pummelo, and was separated from pummelo in 1830 (Webber, 1943). The name was given due to bearing habit just like a cluster of grapes. Shamber and Ray Ruby are the most popular pigmented grapefruit cultivars grown in Pakistan. Pakistan is characterized by a dry warm subtropical climate; making grapefruit prospers, due to its high heat requirements (Morton, 1987). It contributes important nutrients to the diet due to the presence of vitamins A, vitamin C, folic acid, potassium and dietary fiber (Mukherjee, 1997). Grapefruit is considered as preventive against cancer due to rich source of flavonoids and limonoids (Tanaka *et al*., 2000). It is also rich source of naringin, narirutin, and poncerin. Moreover, phytochemicals present in grapefruit act as antioxidants, anti-inflammatory, reduces the cholesterol level and modulates the immune system (Kuo, 1996). These phytochemicals in grapefruit are considered as very important for human body due to their medicinal properties . Phytochemicals constitute one of the most numerous and widely distributed groups substances in the plant kingdom. Flavonoids present in citrus fruit have been extensively studied for antioxidative, anti-cancer, anti-viral, anti-inflammatory activities, capillary fragility, and inhibition of human platelet aggregation of cell during stress in body (Burns and Albrigo, 1998). Therefore, it is important that grapefruit should be harvested and consumed, when it has maximum quantity of these phytochemicals. The grapefruit produced in Pakistan are often of poor quality because growers start to harvest the fruit in early months (July and August) at immature fruit stage. At this stage, phytochemicals are not well developed, and these immature fruits are also sensitive to physiological disorders. Worldwide, efforts have been made to control these problems and to maintain the optimum quality, freshness and minimize the losses during storage (Bajwa and Anjum, 2007). A little information is available about the effects of early and delayed harvesting on these compounds. Furthermore, the harvesting time affects the accumulation of phytochemicals such as TA (total antioxidans), TC (total carotenoids), TL (total limonins), TGL (total glycoside limonins), and TPC (total phenolic contents) and TFC (total flavonoids contents) (Ahmad *et al*., 1992). It has been found that these phytochemicals were found in abundance at the later harvesting times in Valencia oranges (Pekmezci et *al*., 1995). Oliver *et al*. (2004) studied the effects of various

harvesting dates and storage temperatures (6-10°C) on qualitative characters of grapefruit. It was observed that late harvesting of grapefruit showed reduction in titratable acidity (TA) and total soluble solids (TSS) at 6-10°C, and showed no chilling injury after 30 days of storage. Late fruit harvesting reduces the blooming and fruit yield approximately 50% with low quality of Valencia oranges in next year (Hilgemen*et al.*, 1976; Oliver *et al*., 2004). He further reported that ascorbic acid increased slowly until February and then decreased. The objective of the current study was to investigate the optimum harvesting times (harvest maturity) regarding their health promoting compounds .

MATERIALS AND METHODS

***Plants sampling*:**Thirty uniform and healthy grapefruit trees grafted on rough lemon rootstock were selected at Orange Research Institute, Sargodha, (latitude 32° 03' N and longitude 72° 40' E), Punjab, Pakistan. Five uniform 12-year old grapefruit cvs. Ray Ruby and Shamber trees grafted onto sour orange rootstock at 6m × 6m spacing were used. Each tree represented as a replicate unit. Fruits were harvested at different time's viz. 1st September, 1st October, 1st November, 1st December and 1st January during the year 2010-11 and 2011-12 respectively. Fruits were randomly harvested from selected trees with fruit clipper and brought to the Pomology Laboratory Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan.

***Chemicals required for phytochemicals*:** Folin-Ciocalteu reagent, methanol, ethanol, dichloromethane, acetone, hexane and ethyl acetate 2,2-diphenyl-1-picrylhydrazyl (DPPH), β-carotene, linoleic acid (LA), Tween 20, gallic acid, sodium carbonate, ammonium acetate, sodium dihydrogen phosphate, glacialacetic acid, 5-methylphenazinium methosulphate (PMS), 2, 4-dinitrophenylhydrazine (2, 4-DNPH), hydrogen peroxide were purchased from E. Merck (Darmstad, Germany). Deionised water was obtained from an in-house Milli-Q water purification system (Millipore, Bedford, MA, USA).

Phytochemicals analysis of grape fruit:

***Determination of total flavonoid contents*:** Flavonoids were determined by the method of Kim *et al*. (2003).

***Determination of total phenolic compounds and total antioxidants*:** Total antioxidants activities of the grapefruit juice were assessed by measuring their scavenging abilities to 2, 2-diphenyl-1-picrylhydrazyl stable radicals as described by Amira *et al.* (2012). Total phenolic contents (TPC) were calculated by using Folin-Ciocalteu reagent method as reported .

***Extraction and determination of total limonin and total glucoside limonin contents*:** Total limonin and total glycoside limonin were calculated according to the method of Breksa*et al.* (2004).

***Determination of pectin contents*:** The extraction of pectin contents from the fruit peel of grapefruit were studied by following the method .

***Determination of total carotenoids*:** Total carotenoids contents were estimated according to the method of Lichtenthaler and Buschmann (2001).

***Total number of oil glands*:** Total number of oil glands was calculated according to the method of Turrel (1946) by using the following formula. Total gland number = gland density x fruit surface area/180 mm^2

***Fruit firmness*:**Fruit firmness was measured with the help of penetrometer and expressed in Nm^2.

***Total soluble solids*:**Total soluble solids of juice were recorded by using digital hand refractometer (Atago, RX 5000 and Japan).

***Ascorbic acid*:**Five mL of aliquot (containing 10 mL of juice and 90 mL of 0.4% oxalic acid solution) was titrated against 2, 6-dichlorophenolindophenol as an indicator by using the method of Ruck (1961)

***Sugars (total sugars, reducing and non-reducing sugars)*:** Sugars were estimated according to the method of Hortwitz (1960). Reducing sugars were titrated against Fehling's A and B solutions by using methylene blue as an indicator until brick-red color appear as end point. For total sugars, juice samples were first acid hydrolyzed and then titrated by the method described above.

***Statistical analysis*:** Collected data were statistically analyzed using computer software MSTAT-C. The treatments and replications were applied Complete Randomized Design (CRD) under factorial arrangement. Analysis of variance was used to test the significance of variance. While difference among treatments means were compared using LSD test (P=0.05) (Steel *et al*., 1997).

RESULTS

***Total phenolic contents (mg GAE/100 g)*:** Total phenolic contents in fruits showed statistically significant differences regarding the effects of harvesting times and varieties while interaction between them was found non-significant during the both years (Table 1). Fruits harvested in the month of December showed higher total phenolic compounds (172.25 and 176.95 mg GAE/100g) followed by January and November where total phenolic compounds were 147.47 ,150.30, 135.98 and 145.95 mg GAE/100 g during the 2010-11 and 2011-12 respectively. Lower TPC values (101.49 and 101.74 mg GAE/100g) were noted in fruits which were harvested in the month of November during the both years.. The fruits of Shamber showed higher TPC of 144.39 and 151.44 mg GAE/100g as compared to the fruits of Ray Ruby where TPC were 125.72 & 128.22 mg GAE/100g during the both years..

***Total antioxidants (% DPPH inhibition)*:**Statistically significant differences were found for harvesting times and

varieties while their interaction showed non-significant results on total antioxidants in fruits during the both years (Table 2). Higher antioxidants activities (76.36 & 75.08%) were noted when fruit harvested in December followed by January and November during the both years.. However, lower antioxidants (30.52 & 28.66%) were recorded in September during the both years. The Ray Ruby fruit showed lower antioxidants (54.94 & 53.45%) as compared to Shamber fruit (60.98 & 58.53%) during the both experimental years.

***Total flavonoids contents (mg CEQ/100 g)*:** Effect of harvesting times and varieties showed statistically significant results for total flavonoid contents in fruits during the both years, while interaction between them was found non-significant (Table 3). Total flavonoid contents were found in higher amount (65.28 & 69.16 mg CEQ/100 g) during December followed by January and November (58.00, 63.76 & 54.47, 57.50 mg CEQ/100 g) during the both years. Total flavonoids were noted lower (23.19 & 29.06 mg CEQ/100 g) in September during the both years. Shamber fruit showed higher TFC (49.49 & 56.35 mg CEQ/100 g) than Ray Ruby, where TFC were 44.92 & 48.13 mg

Table 1. Effect of different harvesting times on the total phenolic contents (mg GAE/100 g) in Ray Ruby and Shamber fruits.

Harvesting times	2010-2011			2011-2012		
	Ray Ruby	Shamber	Mean	Ray Ruby	Shamber	Mean
1st Sep.	90.59	112.40	101.49 c	97.70	105.78	101.74d
1st Oct.	111.25	125.06	118.15c	119.65	128.73	124.19c
1st Nov.	128.99	142.80	135.89b	133.33	158.77	145.95b
1th Dec.	159.36	185.14	172.25a	153.80	200.10	176.95a
1st Jan.	138.40	156.14	147.47b	136.80	163.81	150.30b
Means	125.72b	144.39a		128.22 b	151.44 a	
LSD value	Varieties = 10.64, Harvesting times = 16.82, Interaction = NS			Varieties = 11.01, Harvesting times = 17.41, Interaction = NS		

Mean sharing same letter in row or column are non-significant at 5% probability level (LSD

Table 2. Effect of different harvesting times on the total antioxidants activities (%DPPH inhibition) in Ray Ruby and Shamber fruits.

Harvesting times	2010-2011			2011-2012		
	Ray Ruby	Shamber	Mean	Ray Ruby	Shamber	Mean
1st Sep.	28.40	32.65	30.52d	22.29	35.03	28.66d
1st Oct.	44.76	49.73	47.24c	50.17	44.73	47.45c
1st Nov.	59.54	65.37	62.45b	59.50	63.83	61.67b
1th Dec.	72.47	80.26	76.36a	72.14	78.03	75.08a
1st Jan.	69.54	76.91	73.22a	63.17	71.03	67.10b
Means	54.94b	60.98a		53.45b	58.53a	
LSD value	Varieties = 3.26, Harvesting times = 5.16, Interaction = NS			Varieties = 4.44, Harvesting times = 7.07, Interaction = NS		

Mean sharing same letter in row or column are non-significant at 5% probability level (LSD)

Table 3. Effect of different harvesting times on the total flavonoids contents (mg CEQ/100 g) in Ray Ruby and Shamber fruits.

Harvesting times	2010-2011			2011-2012		
	Ray Ruby	Shamber	Mean	Ray Ruby	Shamber	Mean
1st Sep.	19.29	27.09	23.19d	26.09	32.03	29.06e
1st Oct.	31.09	39.10	35.10c	35.40	48.06	41.73d
1st Nov.	52.94	56.00	54.47b	60.24	60.24	57.50c
1th Dec.	64.76	65.80	65.28a	74.00	74.00	69.16a
1st Jan.	56.54	59.47	58.00b	67.43	67.43	63.76b
Means	44.92b	49.49a		48.13b	56.35a	
LSD value	Varieties = 2.65, Harvesting times = 4.20, Interaction = NS			Varieties = 2.62, Harvesting times = 4.14, Interaction = NS		

Mean sharing same letter in row or column are non-significant at 5% probability level (LSD)

CEQ/100 g during the both years..

***Total carotenoids contents (mg/100 g)*:**Total carotenoids in fruits showed significant differences regarding the effects of harvesting times and varieties while their interaction did not differ significantly during the both years (Table 4). Total carotenoids were high (20.05 & 20.76 mg/100g) in month of December as compared to January, November, October and September during the both years.. Whereas, total carotenoids (9.53 & 10.88 mg/100 g) were observed low in September during the both years. Shamber fruits showed high total carotenoids (16.29 & 16.38 mg/100 g) than Ray Ruby (15.18 & 15.23 mg/100 g).

***Total pectin contents (%)*:** Statistically significant differences were found for harvesting times while varieties and their interaction showed non-significant results regarding total pectin contents in fruits during the both years (Table 5). Fruits harvested in December showed higher total pectin contents (10.96 & 10.51%) as compared to fruits harvested in January, November, October and September during the first and second season.. Low total pectin contents (4.73 & 5.00%) were found in September during the both years. Total pectin contents were observed high (7.89 & 8.23%) in Shamber fruits as compared to Ray Ruby (7.78 &7 8.11%) during the both study years.

***Total limonin contents (µg/mL)*:**Effects of harvesting times, varieties and their interaction were found statistically significant regarding the total limonin contents in fruits during the both years (Table 6). Higher amounts of total limonin contents (14.88, 13.13 & 14.44, 12 32 µg/mL) were recorded in fruits harvested in September followed by October, November, December and January during the first and second season. While, lower total limonin contents (9.29,

Table 4. Effect of different harvesting times on the total carotenoids (mg/100 g) in Ray Ruby and Shamber fruits.

Harvesting times	2010-2011			2011-2012		
	Ray Ruby	Shamber	Mean	Ray Ruby	Shamber	Mean
1st Sep.	8.84	10.22	9.53e	9.77	11.99	10.88d
1st Oct.	13.72	14.91	14.31d	14.25	14.95	14.60c
1st Nov.	16.59	17.02	16.80c	15.80	15.50	15.65c
1th Dec.	19.03	21.07	20.05a	19.46	22.07	20.76a
1st Jan.	17.70	18.26	17.98b	16.89	17.40	17.14b
Means	15.18b	16.29a		15.23b	16.38a	
LSD value	Varieties = 2.65, Harvesting times = 4.20, Interaction = NS			Varieties = 2.62, Harvesting times = 4.14, Interaction = NS		

Mean sharing same letter in row or column are non-significant at 5% probability level (LSD)

Table 5.Effect of different harvesting times on the total pectin contents (%) in Ray Ruby and Shamber fruits.

Harvesting times	2010-2011			2011-2012		
	Ray Ruby	Shamber	Mean	Ray Ruby	Shamber	Mean
1st Sep.	4.68	4.78	4.73d	4.98	5.03	5.00d
1st Oct.	6.14	6.28	6.21c	7.22	7.35	7.28c
1st Nov.	8.47	8.54	8.51b	7.65	7.74	7.69b
1th Dec.	10.88	11.04	10.96a	10.43	10.58	10.51a
1st Jan.	8.47	8.79	8.67b	10.29	10.45	10.37a
Means	4.68	4.78	4.73d	4.98	5.03	5.00d
LSD value	Varieties = NS, Harvesting times = 0.323, Interaction = NS			Varieties = NS, Harvesting times = 0.196, Interaction = NS		

Mean sharing same letter in row or column are non-significant at 5% probability level (LSD)

Table 6. Effect of different harvesting times on the total limonoids contents (µg/mL) in Ray Ruby and Shamber fruits.

Harvesting times	2010-2011			2011-2012		
	Ray Ruby	Shamber	Mean	Ray Ruby	Shamber	Mean
1st Sep.	4.68	4.78	4.73d	4.98	5.03	5.00d
1st Oct.	6.14	6.28	6.21c	7.22	7.35	7.28c
1st Nov.	8.47	8.54	8.51b	7.65	7.74	7.69b
1th Dec.	10.88	11.04	10.96a	10.43	10.58	10.51a
1st Jan.	8.47	8.79	8.67b	10.29	10.45	10.37a
Means	4.68	4.78	4.73d	4.98	5.03	5.00d
LSD value	Varieties = NS, Harvesting times = 0.323, Interaction = NS			Varieties = NS, Harvesting times = 0.196, Interaction = NS		

Mean sharing same letter in row or column are non-significant at 5% probability level (LSD)

8.67 & 9.66, 9.14 µg/mL) were recorded in fruits harvested in December in both cultivars. Ray Ruby showed higher limonin contents (11.88 &11.93 µg/mL) as compared to Shamber where total limonin contents were 10.77 & 10.96 µg/mL during the both years..

***Total glycoside limonin contents (µg/mL)*:**Total glycoside limonin contents (TGLC) in fruits were significantly different regarding the effects of harvesting times and varieties while interaction between them was found non-significant during the both years (Table 7). Fruits harvested in the month of December showed high total glycoside limonin contents (202.19 & 197.18 µg/mL) followed by January and November where TGLC were 194.54, 191.74 & 188.27, 188.01 µg/mL during the first and second season, respectively. However, lower amount of TGLC (174.39 &171.48 µg/mL) was noted in fruits harvested in September during the both years. The fruits of Shamber showed higher TGLC (191.07 & 190.23 µg/L) as compared to Ray Ruby where TGLC was 185.71 & 181.43 µg/mL during the both experimental years..

***Number of oil glands (180 mm^2)*:**Statistically significant differences were found regarding the harvestingtimes , varieties and their interaction on oil glands in fruits of Ray Ruby and Shamber during the both years (Table 8). Fruits harvested in the month of December showed high oil glands (10625, 8440 & 10291, 7989 180 mm^2) as compared to fruits harvest in other months (January, November, October & September) in both Ray Ruby and Shamber during the first and second season.. While, minimum oil glands of 6893, 5856 & 6909, 6000 180 mm^{-2} were recorded in fruits

Table 7. Effect of different harvesting times on the total glycoside limonin contents (µg/mL) in Ray Ruby and Shamber fruits.

Harvesting times	2010-2011			2011-2012		
	Ray Ruby	Shamber	Mean	Ray Ruby	Shamber	Mean
1st Sep.	172.07	176.70	174.39e	169.82	173.13	171.48d
1st Oct.	181.30	183.78	182.54d	178.45	182.99	18072c
1st Nov.	186.14	190.41	188.27c	183.01	193.02	188.01b
1th Dec.	197.97	206.40	202.19a	190.98	203.38	197.18a
1st Jan.	191.04	198.04	194.54b	184.87	198.62	191.74ab
Means	185.71b	191.07a		181.43b	190.23a	
LSD value	Varieties = 2.90, Harvesting times = 4.59, Interaction = NS			Varieties = 3.64, Harvesting times = 5.76, Interaction = NS		

Mean sharing same letter in row or column are non-significant at 5% probability level (LSD)

Table 8.Effect of different harvesting times on the oil glands (180 mm^{-2}) in Ray Ruby and Shamber fruits.

Harvesting times	2010-2011			2011-2012		
	Ray Ruby	Shamber	Mean	Ray Ruby	Shamber	Mean
1st Sep.	6893e	5856f	6374.30d	6909e	6000f	6893e
1st Oct.	7779cd	7000e	7389.20c	7780cd	7215de	7779cd
1st Nov.	7967cd	7489de	7728.30c	7922c	7558cd	7967cd
1th Dec.	10625a	8440c	9532.70a	10291a	7989c	10625a
1st Jan.	9624b	7966cd	8795.00b	9520b	7818cd	9624b
Means	8577.70a	7350.10b		8484.40a	7317.90b	8577.70a
LSD value	Varieties = 301.79, Harvesting times = 477.17, Interaction = 674.82			Varieties = 281.82, Harvesting times = 445.59, Interaction = 630.16		

Mean sharing same letter in row or column are non-significant at 5% probability level (LSD)

Table 9.Effect of different harvesting times on the fruit firmness (Nm^2) in Ray Ruby and Shamber fruits.

Harvesting times	2010-2011			2011-2012		
	Ray Ruby	Shamber	Mean	Ray Ruby	Shamber	Mean
1st Sep.	0.60	0.56	0.58d	0.58	0.59	0.59d
1st Oct.	0.68	0.65	0.66c	0.68	0.65	0.60c
1st Nov.	0.71	0.69	0.70c	0.77	0.77	0.72b
1th Dec.	0.87	0.86	0.87a	0.82	0.82	0.85a
1st Jan.	0.76	0.78	0.77b	0.79	0.79	0.79 ab
Means	0.72a	0.71b		0.73a	0.73b	
LSD value	Varieties = NS, Harvesting times = 0.036, Interaction = NS			Varieties = NS, Harvesting times = 0.058, Interaction = NS		

Mean sharing same letter in row or column are non-significant at 5% probability level (LSD)

harvested in November during the two successive experimental years..

***Fruit firmness (Nm^2)*:** Firmness in fruits of Ray Ruby and Shamber showed significant differences regarding the harvesting times, while non-significant difference was recorded in varieties and their interaction during the both years (Table 9). Lower fruits firmness (0.582 & 0.593 Nm^2) was examined in fruits which were harvested earlier in the month of November during the both years. High firmness of 0.871 & 0.845 Nm^2 was recorded in fruits, which were harvested in the month of December, and then gradually decreased with the delay in fruit harvesting up to January where firmness in fruits was 0.770 and 0.792 Nm^2 in both cultivars during the first and second season..

***Total soluble solids (°Brix)*:**Total soluble solids in fruits showed significant differences regarding the harvesting times and varieties, while their interaction was non-significant during the both years (Table 10). Fruits harvested in the month of December showed high TSS contents (9.66 & 9.81 °Brix) followed by January and November. While, low TSS contents (5.46 & 5.56 °Brix) were noted in fruits which were harvested in November during the both years, respectively.

***Ascorbic acid contents (mg/100 g)*:**Statistically significant results were found for the effects of harvesting times, while varieties and their interaction showed non-significant differences for ascorbic acid contents in fruits during the both years (Table 11). Higher amounts of ascorbic acid contents (40.27 & 40.44 mg/100 g) were noted in fruits which were harvested in December followed by the months of January and November and these were statistically at par with each other during first and the second season, respectively. Minimum ascorbic acid contents (30.59 & 30.39 mg/100 g) were found in fruits harvested in November during the both years.

***Total sugars contents (%)*:** Total sugar contents in fruits showed statistically significant differences regarding the harvesting times and varieties, while their interaction was non-significant during the both years (Table 12). Shamber produced high total sugars (6.11 & 6.27%) as compared to Ray Ruby (6.01 & 6.16%) during the both years. Fruits harvested in the month of December had high total sugar contents (6.99 & 6.96%) in fruits followed by January and November where total sugars were 6.48, 6.71 & 6.33, 6.52% during the both years, respectively. Lower amount of total sugar contents (4.28 & 4.44 %) were noted in fruits harvested in November during both years.

***Reducing sugar contents (%)*:**Higher amounts of reducing sugar contents (5.66 & 5.33%) were recorded in fruits which were harvested in the month of December followed by fruits harvested in January and November and these were statistically at par (Table 13). Whereas, lower amount of

Table 10. Effect of different harvesting times on the total soluble solids (°Brix) in Ray Ruby and Shamber fruits.

Harvesting times	2010-2011			2011-2012		
	Ray Ruby	Shamber	Mean	Ray Ruby	Shamber	Mean
1st Sep.	5.03	5.89	5.46d	5.32	5.80	5.56e
1st Oct.	6.69	6.83	6.76c	6.40	7.15	6.78d
1st Nov.	8.34	8.65	8.50b	8.48	8.96	8.72c
1th Dec.	9.43	9.88	9.66a	9.63	9.99	9.81a
1st Jan.	8.51	8.75	8.63b	9.10	9.25	9.18b
Means	7.60b	8.00a		7.79b	8.23a	
LSD value	Varieties = 0.160, Harvesting times = 0.253, Interaction = NS			Varieties = 0.192, Harvesting times = 0.304, Interaction = NS		

Mean sharing same letter in row or column are non-significant at 5% probability level (LSD)

Table 11. Effect of different harvesting times on the ascorbic acid contents (mg/100 g$^-$) in Ray Ruby and Shamber fruits.

Harvesting times	2010-2011			2011-2012		
	Ray Ruby	Shamber	Mean	Ray Ruby	Shamber	Mean
1st Sep.	30.56	30.63	30.59d	30.62	30.29	30.45d
1st Oct.	33.67	34.06	33.86c	33.37	34.21	33.79c
1st Nov.	36.73	36.98	36.86b	36.76	37.47	37.11b
1th Dec.	39.65	40.89	40.27a	40.41	40.48	40.44a
1st Jan.	37.44	38.22	37.83b	37.62	38.00	37.81b
Means	35.61b	36.15a		35.75a	36.09 a	
LSD value	Varieties = NS, Harvesting times = 1.31, Interaction = NS			Varieties = NS, Harvesting times = 1.27, Interaction = NS		

Mean sharing same letter in row or column are non-significant at 5% probability level (LSD)

Table 12. Effect of different harvesting times and varieties on the total sugars (%) in Ray Ruby and Shamber fruits.

Harvesting times	2010-2011			2011-2012		
	Ray Ruby	Shamber	Mean	Ray Ruby	Shamber	Mean
1st Sep.	4.21	4.35	4.28e	4.38	4.51	4.44d
1st Oct.	6.14	6.31	6.22d	6.37	6.51	6.44c
1st Nov.	6.23	6.43	6.33c	6.42	6.62	6.52c
1th Dec.	7.03	6.95	6.99a	6.95	6.97	6.96a
1st Jan.	6.43	6.53	6.48b	6.66	6.75	6.71b
Means	6.01b	6.11a		6.16b	6.27a	
LSD value	Varieties = 0.066, Harvesting times = 0.104, Interaction = NS			Varieties = 0.065, Harvesting times = 0.102, Interaction = NS		

Mean sharing same letter in row or column are non-significant at 5% probability level (LSD)

Table 13. Effect of different harvesting times on the reducing sugars (%) in Ray Ruby and Shamber fruits.

	2010-2011			2011-2012		
	Ray Ruby	Shamber	Mean	Ray Ruby	Shamber	Mean
1st Sep.	3.11	3.22	3.16d	3.02	3.17	3.09d
1st Oct.	4.80	4.95	4.87c	4.34	4.58	4.46c
1st Nov.	4.90	5.07	4.99 bc	4.49	4.72	4.61bc
1th Dec.	5.59	5.73	5.66a	5.26	5.41	5.33a
1st Jan.	5.04	5.16	5.10b	4.62	4.91	4.76b
Means	4.69b	4.82a		4.34b	4.56a	
LSD value	Varieties = 0.126, Harvesting times = 0.200, Interaction = NS			Varieties = 0.103, Harvesting times = 0.163, Interaction = NS		

Mean sharing same letter in row or column are non-significant at 5% probability level (LSD)

Table 14. Effect of different harvesting times on the non reducing sugars (%) in Ray Ruby and Shamber fruits.

	2010-2011			2011-2012		
	Ray Ruby	Shamber	Mean	Ray Ruby	Shamber	Mean
1st Sep.	1.260	1.226	1.243d	1.306	1.256	1.281d
1st Oct.	1.470	1.410	1.440c	1.536	1.476	1.506c
1st Nov.	1.663	1.593	1.628b	1.570	1.543	1.556bc
1th Dec.	2.133	1.993	2.063a	2.043	1.866	1.955a
1st Jan.	1.723	1.650	1.686b	1.630	1.590	1.610b
Means	1.650a	1.574b		1.617a	1.546 b	
LSD value	Varieties = 0.061, Harvesting times = 0.097, Interaction = NS			Varieties = 0.099, Harvesting times = 0.219, Interaction = NS		

Mean sharing same letter in row or column are non-significant at 5% probability level (LSD)

reducing sugar contents of 3.16 & 3.09% were noted in fruits harvested in September during the both years,. Regarding the response of cultivars, Shamber showed higher reducing sugar contents of 4.82 & 4.56% as compared to the fruits of Ray Ruby where reducing sugar contents were 4.69 & 4.34% during the first and second season.

***Non reducing sugars (%)*:**Fruits harvested in the month of December showed higher amounts of non-reducing sugars (2.063 & 1.955%) followed by fruits harvested in January and November where non-reducing sugars were 1.686, 1.610 & 1.628, 1.556% during the both years, respectively. While, low amount of non-reducing sugars (1.243 & 1.281%) were noted in fruits harvested in September during the both years, respectively. Fruits of Ray Ruby showed higher non-reducing sugars (1.650 & 1.617%) as compared to Shamber fruits where non-reducing sugars were 1.574 & 1.546% during the first and second season, respectively (Table 14).

DISCUSSION

Harvesting time is a key component, and affects the external and internal characteristics of the fruit. The results of this study revealed that maximum phenolic compounds were in fruits harvested in December as compared to other harvesting times . Early (Sep, Oct and Nov.) and late harvested (Jan) fruits showed lower amounts of total

phenolic compounds. These results are in agreement with Andreotti *et al.* (2008) and Remorini *et al.* (2008) who observed significant reduction in phenolic compounds in early and late harvested citrus fruits (lemon and limes). They reported that lower phenolic compounds were due to lower level of ethylene which was measured in early and late harvested fruits, because ethylene is the precursor of these compounds. High antioxidants, carotenoids, flavonoids and pectins were observed in fruits harvested in December, which also indicates that such compounds are directly proportional to the level of optimum maturation processes. Grierson and Tucker (1983) and Goodner*et al.* (2001) noted increased activities of enzymes at optimum maturity. Callahan *et al*. (2004) also reported that during maturation process, enzymatic activities enhanced the level of pectin esterase. Maximum level of limonin and total glycoside limonin in these fruits were due to more accumulation of natural compounds which are stored at optimum level in mature fruits (Goodner*et al.,* 2001; Lee and Castle, 2001). December harvested fruits showed maximum oil glands and this was due to optimum size of fruits. The results of Turner *et al*. (1998) support this reason who reported that early and late harvesting times showed lower oil glands on the fruit surface due to improper fruit size and structure. Maximum oil glands were found in Ray Ruby fruits than Shamber during both years of experiment. It may be due to the genetic and climatic variations. High total soluble solid contents and lower acidity at optimum maturity also supports the findings of Rizzolo and Eccher (2006) who reported that total soluble solid content increased and moisture content decreased up to the maximum maturity of grapefruit .

***Conclusion*:** In conclusion, health promoting substances increased with the advancement of maturity but these started to decline after optimum stages. Moreover, grapefruits have showed the highest values of all the essential phytochemicals (TPC, TA, TP, TC, TF, TL and TGL) and other quality related parameters (TSS, lower fruit firmness, ascorbic acid, TS, RS and NRS) in the month of December. Therefore, it is recommended that fruits of these cultivars should be harvested in this month for obtaining maximum benefit for human health.

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