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Evaluation of Cultivated Lime and Lemon Cultivars in Southern Iran for Some Biochemical Compounds

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ABSTRACT

There were no significant differences in the contents of total dietary fiber in the cultivars of lime (*Citrus aurantifolia* Swing cvs. 'Persian' and 'Roodan') and lemon (*Citrus limon* L. Burm cvs. 'Lisbon' and 'Meyer') tested but the contents of total dietary fiber in peel were significantly higher than pulp ($P < 0.05$). The content of total polyphenols showed significant differences in each part and in each cultivar. The highest total phenolic content was in leaves which ranged from 99.75 ± 0.68 to 129.42 ± 0.48 mg gallic acid/100 g DW than in green and yellow peels, petals, pulp and seed of all cultivars. Carotenoid and pectin contents were significantly different in the different parts of cultivars, significantly higher in fruit peel than in pulp. Lemon juice had higher antioxidant activity, TSS and pH than limes but lower ascorbic acid, EC and titratable acidity than lime juice. In conclusion, the peels of all cultivars were rich in dietary fiber, pectin and total carotenoid. All fruit parts of lemon cultivars had highest polyphenolic content. Peel and pulp of lime fruits showed highest pectin content.

Keywords: carotenoid, lemon, lime, pectin, polyphenol

Abbreviations: **A**, absorption; **AoA**, antioxidant activity; **DPPH**, 2,2-diphenyl-1-picrylhydrazyl; **DW**, dry weight; **EC**, electrical conductivity; **FW**, fresh weight; **SW**, sample weight; **TA**, tiratable acidity; **TSS**, total soluble solid; **TV**, total volume

INTRODUCTION

Among various parts of plants, fruit is important as a natural antioxidant compound that has an important role in free radical-scavenging activities. These compounds include polyphenol, carotenoids, flavonoids and other compounds (Morel *et al.* 1994).

Citrus are among the most important fruits in tropical and subtropical regions (Hvarleva *et al.* 2008). Lime and lemon having differences in some characters, but because their most important similarity is their high level of titratable acidity, people often cannot recognize them and they are often both termed lemon. Lime (*Citrus aurantifolia* Swing) and lemon (*Citrus limon* L. Burm) are two species of the *Citrus* genus that, after oranges and mandarins, are ranked third in production and are cultivated in many countries. The value of citrus fruits in providing nutrients and medicinal components has been recognized since ancient times (Khan 2007). These fruits were mainly used in daily consumption and in juice production. Peripheral products of citrus fruit such as essential oil and pectin of fruit peel are used in the cosmetic and pharmaceutical industries (Khan 2007; Ladaniya 2008). By-products from citrus processing are used to produce other valuable compounds such as seed oil, pectin and other compounds which are used in the food, drug and cosmetics industries (Rouse and Crandall 1976; Bocco *et al.* 1998).

In Iran and some Middle East countries, whole lime fruit is dried and is used in some foods. The flowers are also used in tea.

Several studies about the evaluation of antioxidants in different parts of citrus plants have been realized, but this information is limited to some species of citrus and the differences between varieties in each species has not been investigated. Nowadays, the need for preservatives in the food industry is completely evident; one of these preservatives are antioxidant compounds, which are often used in synthetic form. On the other hand, synthetic antioxidants may be toxic and carcinogenic and their use in the food industry is prohibitive and the use of the natural antioxidant compounds is suggested (Louli *et al*. 2004; Rehman 2006; Garau *et al.* 2007). Citrus fruits possess high antioxidant capacity, possibly due to the amount of vitamin C, phenolic compounds, flavonoids and caretonoids. Most citrus juice has similar nutritional value but there are some differences, especially in the amount of vitamin C, antioxidant activity and citric acid (Rapisarda *et al*. 1999; Saidani and Marzouk 2003). Therefore, using citrus fruits is recommended from a health point of view.

MATERIALS AND METHODS

Plant material and sample preparation

This study was attempted to evaluate some biochemical compounds extracted from different parts of fruits in four cultivars of limes and lemons cultivated in southern parts of Iran. Two lime cultivars (cvs. 'Persian' and 'Roodan') and two lemon cultivars (cvs. 'Lisbon' and 'Meyer') were used for the experiment. 'Roodan' lime is a local cultivar in Southern Iran which has unique characteristics such as thornless stems and branches. These characteristics are important since the harvesting of fruits is made easier. Several fruits of each cultivar were harvested randomly from different parts of the trees at local farms. All parts of fruits were separated by hand into pulp, peel and seed. Some samples were used fresh but were dried and stored at -80°C for further use.

Total polyphenol content

The leaf, green peel, yellow peel, pulp, seed and petal samples were used to measure the phenolic compounds. Mature green fruits were collected to evaluate their phenolic compounds in peel. Phenolic compounds were extracted according to Vinson *et al.* (2001). All parts were dried in an oven at between 35 and 40°C for

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72 h. Dry samples were powdered by milling. Total phenolic compounds were extracted by percolation with methanol (50%) and were measured according to a procedure as suggested by Folin-Ciocalteau (Singleton and Rossi 1965).

Pectin content and total dietary fiber

For measuring the pectin content, 25 g of fresh sample (peel or pulp) was boiled with 400 ml distilled water for 1 h; evaporated water was replaced by distilled water. After cooling, the mixture was filtered through Whatman No. 4 filter paper following which 10 ml of NaOH (1 N) was added and kept overnight in room temperature (25°C). Then, 50 ml of acetic acid (1N) was added and left for 5 min, 25 ml $CaCl_2$ (1N) was added and the mixture was placed for 1 h at room temperature (25°C) then boiled for 1 min. Two sheets of Whatman filter paper No. 4 were washed with deionized water and dried in oven in 100°C for 2 h. The filter paper sheets were weighted and then the solution was passed through them. To remove chlorine ions, residues were washed with deionized water. A few drops of silver nitrate solution were added and then filter paper sheets were placed in an oven to be dried. After drying, they were weighed, and the difference between primary and secondary weight of paper equaled the dry weight of pectin (Rangana 1977). Total dietary fiber was measured according to AOAC (1984).

Total carotenoids

The carotenoid content in the pulp and peel of fruits was determined. The carotenoids were extracted by hexane: ethanol (9: 1). 1 g of fresh sample (pulp or peel) was weighed then crushed using 5 ml of extraction solution on ice followed by centrifuging at 10,000 rpm at 4°C. The supernatant was removed and the remaining extraction solution was added to the previous extraction. This extraction was repeated until all carotenoid compounds were extracted and the sample became colorless. All supernatants were mixed and then the absorbance of the mixture was measured at 480 nm with a spectrophotometer (Perkin Elmer, Lambda-EZ201, USA) as per the method suggested by Olsson *et al* (2005). Finally, the total carotenoids were obtained using the formula (Gross 1991):

$$\text{Total carotenoids } (\mu g/g) = (TV \times A \times 10^6)/(2500 \times 100 \times SW) \quad (1)$$

Fruits juice characteristics

Fresh juice was extracted with a manual juicer. Ascorbic acid, titratable acidity, pH, total soluble solids (TSS), electrical conductivity (EC) and antioxidant activity were measured. Ascorbic acid was measured according to the protocol of Redox titration using iodine solution (www.outreach.canterbury.ac.nz). Titratable acidity (TA) was measured with 0.1 N NaOH to a final pH of 8.2 and calculated based on citric acid. pH and EC were determined with a pH meter and an EC meter, respectively. TSS was determined with a refractometer (Atago Co., Japan). Antioxidant activity (AA) of fruits juice was determined by the DPPH method according to Moon and Terao (1998). Fruit juice was mixed with 0.9 ml of 100 mM Tris-HCl buffer (pH = 7.4) then 1 ml of DPPH (500 µM in ethanol) was added. The mixture was shaken and left at room temperature in the dark for 30 min. The absorbance of the reaction mixture was measured at 517 nm with a spectrophotometer (Perkin Elmer, Lambda-EZ201). AA was calculated using the following equation (Moon and Terao 1998):

$$AA\ (\%) = (1\text{-}A_{\text{sample (517nm)}} / A_{\text{control (517nm)}}) \times 100$$

Statistical analyses

The experiment was conducted in randomized complete block with three replications. Analysis of variance with general linear means model was done using SAS (v. 9.0, Cary, NC) and where interactions were not significant, main effects were separated by Duncan's multiple range test at $P \leq 0.05$. To verify the statistical significance of all parameters, the values of means ± SD were calculated. Bivariate simple correlation between characters was performed with SPSS software Ver. 10.

RESULTS

Total polyphenols

In all samples the total phenolic contents were higher in leaves, green peel, yellow peel, petals, pulps and seeds. The total polyphenol content in all lemons parts were higher than in limes. There were significant differences in total polyphenol content between all cultivars. In all cultivars green peels had higher polyphenol content than yellow peels. Total polyphenol content in all parts of lemons ranged between 8.23 ± 0.26 and 129.42 ± 0.48 mg/100 g DW and in limes between 4.22 ± 0.15 and 100.54 ± 0.50 mg/100 g DW (**Fig. 1**). There was no significant difference between total polyphenol content of leaves, pulp, petals and seed in the two lime cultivars, but total polyphenol content of green peels was significantly higher than that of yellow peels.

In all cultivars tested the highest amount of total polyphenol content in leaves was observed in cv. 'Lisbon' (129.42 ± 0.48 mg/100 g DW) followed by 'Meyer' lemon (122.04 ± 0.32 mg/100 g DW). Total polyphenol content in the leaves of 'Persian' lime (99.75 ± 0.68 mg/100 g DW) was not significantly different from that of 'Roodan' lime (100.54 ± 0.51 mg/100 g DW), but it was lower than lemon cultivars. There were no significant differences between polyphenol content in the pulp of 'Persian' lime (33.25 ± 0.68 mg/100 g DW) and 'Roodan' lime (31.66 ± 0.41 mg/100 g DW), while the total polyphenol content in the pulp of 'Meyer' lemon (50.61 ± 0.83 mg/100 g DW) was higher than that of 'Lisbon' (44.20 ± 0.31 mg/100 g DW). Also, all parts of each lemon cultivar had a higher amount of total polyphenol content than each lime cultivar. The amount of polyphenol content in the petals of 'Persian' lime (40.72 ± 0.54 mg/100 g DW) and 'Roodan' lime (38.23 ± 0.28 mg/100 g DW) was lower than in 'Lisbon' lemon (50.25 ± 0.82 mg/100 g DW) and 'Meyer' lemon (46.04 ± 0.52 mg/100 g DW). These amounts were close to the amount of total polyphenol content of pulp.

The total polyphenol content of the green peels of 'Persian' lime (60.49 ± 0.35 mg/100 g DW) was significantly higher than that of 'Roodan' lime (56.04 ± 0.41 mg/100 g DW), and also higher than total polyphenol content of yellow peels in these cultivars ['Persian' lime (54.74 ± 0.57 mg/100 g DW), 'Roodan' lime (48.84 ± 0.39 mg/100 g DW)]. The amount of total polyphenols in yellow peels of 'Meyer' lemon (64.72 ± 0.48 mg/100 g DW) was higher than that of 'Lisbon' lemon (60.15 ± 0.52 mg/100 g DW) while that of green peels of 'Meyer' lemon (73.17 ± 0.62 mg/100 g DW) was higher than that of 'Lisbon' lemon (67.51 ± 0.58 mg/100 g DW) (**Fig. 1**).

Dietary fiber and pectin contents

Comparative results of fiber contents in the pulp and peel of all of lime and lemon cultivars is presented in **Fig. 2**. In all cultivars total dietary fiber in peels was higher than in the pulp. There were significant differences in the peel between limes and lemons in terms of total dietary fiber content but not in the pulp of lime and lemon. The range of total dietary fiber content in peel and pulp of limes was 5.01 ± 1.14 and 9.02 ± 0.88 g/100 g DW, respectively, higher than lemons (4.86 ± 1.28 and 7.31 ± 1.07 g/100 g DW; **Fig. 2**). The dietary fiber content in the peels of 'Persian' lime (8.62 ± 1.19 g/100 g DW) was lower than that of 'Roodan' lime (9.02 ± 0.88 g/100 g DW) and this parameter in 'Lisbon' lemon (7.31 ± 1.07 g/100 g DW) and 'Meyer' lemon (7.21 ± 1.16 g/100 g DW) was not significant. The pectin content in the peel and pulp of both lime cultivars were higher than that of lemon cultivars. In all samples the pectin content in peel was higher than that of pulp (**Fig. 3**).

Total carotenoids

Total carotenoids in the fruit peels of all cultivars were

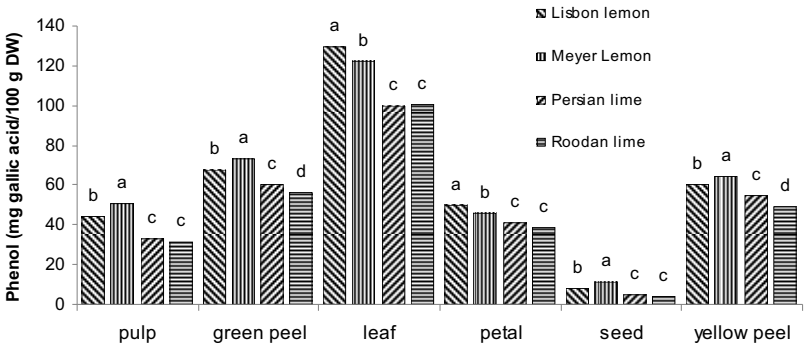

Fig. 1 Total phenolic content in limes and lemons organs. Different letters represent significant differences within each cultivar organs separated independent using Duncan's multiple range test, $P < 0.05$.

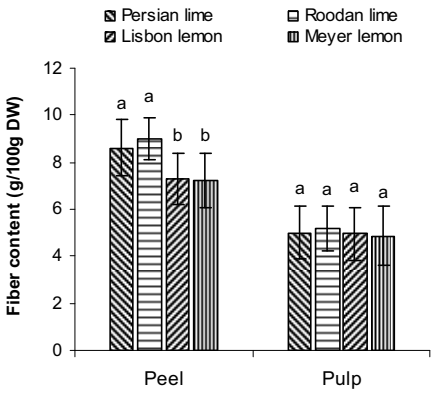

Fig. 2 Content of dietary fiber in dry weight. Data are means ± S.D. of three measurements. Different letters represent significant differences within each cultivar organs separated independent using Duncan's multiple range test, $P < 0.05$.

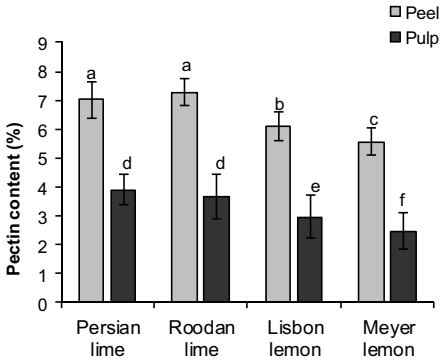

Fig. 3 Content of pectin in fresh weight. Data are means ± S.D. of three measurements. Different letters represent significant differences within each cultivar organs separated independent using Duncan's multiple range test, $P < 0.05$.

Table 1 Total carotenoid contents (µg/g, fresh weight) in peel and pulp of four cultivars of lime and lemon.

Cultivar	Peel	Pulp
Persian lime	267.93 ± 4.49 c	170.73 ± 1.89 g
Roodan lime	251.43 ± 4.27 d	156.6 ± 1.51 h
Lisbon lemon	281.4 ± 2.3 b	193.47 ± 2.72 f
Meyer lemon	294.1 ± 4.3 a	207.67 ± 2.58 e

Data are means ± S.D. of three measurements
Data with different letters are significantly different at $P < 0.05$ using Duncan's multiple range test

significantly higher than in fruit pulp. The amount of total carotenoids in the peels and pulps of four limes and lemons fruits are shown in **Table 1**. The highest level of carotenoids was detected in 'Meyer' lemon and 'Lisbon' lemon peels (294.1 ± 4.3 and 281.4 ± 2.3 µg/g FW) followed by 'Meyer' lemon pulp (207.67 ± 2.58 µg/g FW). All parts of lemon fruits had a higher level of total carotenoids than lime fruits.

Fruit juice characteristics

The amount of vitamin C in 'Persian' lime and 'Roodan' lime fruits was 56.71 ± 0.63 and 41.6 ± 0.41 mg/100 g FW, respectively and in 'Lisbon' lemon and 'Meyer' lemon fruits 38.03 ± 0.75 and 35.2 ± 0.63 mg/100 g FW, respectively (**Table** 2). There were significant differences in the amount of vitamin C between lime and lemon cultivars. The highest amount of vitamin C was in limes cultivars, especially in 'Persian' lime.

There were significant differences in pH, EC, TSS and TA between lime and lemon cultivars (**Table** 2). Lime fruit juice had higher EC and TA values but lower pH and TSS. The range of pH in lime fruit juice was 1.77 ± 0.03 and 1.67 ± 0.02 in 'Persian' and 'Roodan' lime, respectively and in lemon fruit juice, it was 2.61 ± 0.06 and 2.67 ± 0.03 in 'Lisbon' and 'Meyer' lemon, respectively.

The percentage antioxidant activity in lime and lemon cultivars ranged from 32.52 ± 0.67 to 65.87 ± 0.39%, that the antioxidant activity in lemon cultivars was significantly higher than in lime cultivars.

Bivariate simple correlation analysis

Bivariate correlation between two characters showed a relationship that is not considered as a kind of effect but makes it possible to indirectly measure another character (Jonson and Wichern 1988). The results of simple correlation analysis showed the existence of significant positive and negative correlations among some characters (**Table 3**).

Correlation coefficients among dependent variables indicated a relation between carotenoid contents in pulp and polyphenol contents in pulp and peel of fruits (**Table 1**). Antioxidant activity was positively correlated with polyphenol as well as carotenoid content in the peel and pulp. No significant correlation among pH, TA, EC and vitamin was noted; however, the only significant positive correlation between antioxidant activity and pH in fruit juice was measured.

Positive correlations were observed between antioxidant activity and polyphenol content in all parts of fruits such as green peel, yellow peel, pulp and seed, and also between antioxidant activity and carotenoid content in the peel and pulp of fruit. A positive correlation between fiber content in peel and pectin content in peel of fruits was found. There was a positive correlation between pectin content in peel and pulp (r = 0.96) and also between carotenoid content in peel and pulp (r = 0.99).

DISCUSSION

The compounds found in plants have a wide range of physiological effects on human beings and may protect against various diseases, including heart diseases and cancer. Seve-

Table 2 Fruit juice characters of four cultivars of lime and lemon.

Cultivar	pH	TA	TSS	EC	Vit. C	AoA
Persian lime	1.77 ± 0.03 b	7.11 ± 0.05 a	7.87 ± 0.03 b	4933.33 ± 16.67 a	56.71 ± 0.63 a	36.7 ± 0.42 c
Roodan lime	1.67 ± 0.02 b	6.58 ± 0.09 b	7.8 ± 0 b	4783.33 ± 16.67 b	41.6 ± 0.41 b	32.52 ± 0.67 d
Lisbon lemon	2.61 ± 0.06 a	5.46 ± 0.12 c	8.27 ± 0 a	3666.67 ± 16.67 c	38.03 ± 0.75 d	65.87 ± 0.39 b
Meyer lemon	2.67 ± 0.03 a	5.37 ± 0.15 c	8.2 ± 0.03 a	3833.33 ± 44.1 d	35.2 ± 0.63 c	56.3 ± 0.76 a

Data are means ± S.D. of three measurements

Data with different letters are significantly different at $P < 0.05$ using Duncan's multiple range test

TA: Tiratable acidity (%), TSS: Total soluble solid (%), EC: Electrical conductivity (µmho/cm), Vit. C: Vitamin C (mg/ 100 g FW), AoA: Antioxidant activity (%)

Table 3 Bivariate simple correlations between some characters measured in different parts of lime and lemon.

Character	ph- gp	ph-yp	ph-pu	ph-s	ph-pet	ph-l	p-pu	p-pe	C-pu	C-pe	F-pu	F-pe	AoA	pH	TSS	AsA	TA	EC
ph- gp	1																	
ph-yp	0.99**	1																
ph-pu	0.98*	0.95*	1															
ph-s	0.98*	0.96*	0.99**	1														
ph-pet	0.80	0.81	0.81	0.75	1													
ph-l	0.83	0.81	0.87	0.81	0.97*	1												
p-pu	-0.92	-0.88	-0.97*	-0.96*	-0.76	-0.86	1											
p-pe	-0.99**	-0.97*	-0.99**	-0.99**	-0.81	-0.87	0.96*	1										
C-pu	0.99**	0.99**	0.98*	0.98*	0.83	0.85	-0.92	-0.99**	1									
C-pe	0.99**	1.00**	0.95*	0.96*	0.8	0.80	-0.87	-0.96*	0.99**	1								
F-pu	-0.94	-0.97*	-0.86	-0.89	-0.73	-0.70	0.74	0.89	-0.94	-.97*	1							
F-pe	-0.96*	-0.95*	-0.96*	-0.93	-0.93	-0.95*	0.91	0.96*	-0.97*	-0.94	0.88	1						
AoA	0.98*	0.96*	0.99**	0.99*	0.83	0.89	-0.96*	-0.99**	0.98*	0.96*	-0.88	-0.97*	1					
pH	0.92	0.90	0.94	0.90	0.95*	0.98*	-0.92	-0.94	0.93	0.89	-0.81	-0.99**	.095*	1				
TSS	-0.23	-0.24	-0.26	-0.16	-0.76	-0.69	0.24	0.26	-0.28	-0.23	0.18	0.4	-0.30	-0.57	1			
AsA	-0.50	-0.41	-0.64	-0.57	-0.63	-0.77	0.76	0.60	-0.51	-0.39	0.20	0.62	-0.62	-0.70	0.51	1		
TA	-0.82	-0.76	-0.89	-0.84	-0.87	-0.96*	0.93	0.87	-0.82	-0.75	0.61	0.90	-0.89	-0.94	0.55	0.89	1	
EC	-0.88	-0.84	-0.90	-0.93	-0.48	-0.6	0.91	0.89	-0.85	-0.83	0.75	0.75	-0.88	-0.71	-0.16	0.50	0.70	1

* $P < 0.05$, ** $P < 0.01$

Ph-gp: phenolic conent in green peel, ph-yp: phenolic content in yellow peel, ph-pu: phenolic content in pulp, ph-s: phenolic content in seed, ph-pet: phenolic content in petal, ph-l: phenolic content in leaf, p-pu: pectin content in pulp, p-pe: pectin content in peel, C-pe: Carotenoid content in peel, C-pu: Carotenoid content in pulp, F-pu: fiber content in pulp, F-pe: fiber content in peel, AoA: antioxidant activity, TSS: total soluble solid, AsA: ascorbic acid, TA: titratable acidity, EC: electrical conductivity.

ral classes of phytochemicals including monoterpenes, limonoids (triterpenes), flavanoids, carotenoids and phenolic compounds have been isolated from citrus. In recent years, more attention is paid to phenolic compounds of citrus fruits, as they have antioxidant capacity (Wang *et al.* 1996; Rapisarda *et al*. 1999; Gorinstein *et al*. 2004).

Gorinstein *et al* (2001) found that polyphenol content in citrus fruit peel was significantly higher than pulp; also they found the nutrition value of peel of citrus fruits was similar to that of our results. The difference about among of polyphenol in same citrus fruits might be due to difference in environmental conditions of growing (Belitz and Grosch 1999). Further, the level of polyphenol content in green peel of lime and lemon fruits were higher than limes. In fact, the ripening stage might lead to a decrease in phenolic compounds in fruit peels. We found a significant positive correlation between antioxidant activity and phenolic content in pulp. The phenolic content acts as powerful antioxidant in citrus fruits (Gorinstein *et al.* 2007; Khandaker *et al.* 2008). The reason for high levels of polyphenols in petals is not clear; polyphenols are by plants to protect flowers against attack by phytopathogens (Im *et al.* 2008).

Curl and Bailey (1956) and Gorinstein *et al.* (2001) reported that the dietary fiber content in lime and lemon peels were higher than pulp as also found in this experiment. This may be associated with the peel thickness, especially the albedo, in lemon fruits, which is thicker than that of limes.

The peels of four cultivars had higher pectin content than pulp. Maximum content of total pectin was found in 'Roodan' lime and 'Persian' lime peel and also in the pulp of 'Roodan' lime and 'Persian' lime, which had the highest pectin content. Rouse and Atkins (1956) reported that lime peels had a higher pectin content than lemon peels.

The carotenoid content in the peel of limes and lemons fruits was higher than in the pulp. Two lemon cultivars had a higher carotenoid content than lime cultivars.

Lemon fruits juice had higher pH and antioxidant activity than lime juice. Lime fruit juice had higher EC, vitamin C and titratable acidity than lemon fruits. The fruit juice of lime was more sour than lemon; this is related with the content of organic acid in fruit juice such as citric acid and ascorbic acid.

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