



Total phenol, total flavonoid and ascorbic acid content of Iranian commercial orange juice

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ABSTRACT

This study was designed to measure and compare the total phenols, flavonoids and vitamin C contents in four orange juice brands which are commercially available in Iran. Total phenols, flavonoids and ascorbic acid content of 100 samples from four different commercial brands were evaluated by the spectrometric method. The concentration of total phenol in commercial orange juice samples was between 28.39 and 114.20 mg gallic acid equivalent per liter (mg GAE/L). The measured range of total flavonoids was from 12.53 to 32.62 mg quercetin equivalent per liter (mg QE/L) and the content of ascorbic acid in the samples was between 29.95 and 93.08 mg/L. The results showed a significant difference between the four brand's total phenols, flavonoids and vitamin C level ($P < 0.05$). According to the found variation among different studied brands, setting a determined amount for the measured parameters is suggested.

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1. Introduction

Reactive oxygen species (ROS) are chemically reactive molecules like hydrogen peroxide, hydroxyl radical and superoxide anion radical that are produced in body by many enzymatic systems through oxygen utilization (1). These ROS also can be effective as growth regulators (2). Large amount of ROS may cause several conditions such as cardiovascular, cancer and neurodegenerative diseases (3). Therefore, exogenous antioxidants are regularly required to keep a sufficient amount of antioxidants for balancing the ROS (4).

Epidemiological researches have suggested that the utilization of natural antioxidants like fruits and polyphenol-rich foods have defensive effects against the cardiovascular diseases and this protection has been at somewhat imputed to the existence of several components, such as flavonoids, phenolic compounds and vitamins (5).

Antioxidants influence the mechanism of lipid peroxidation, neutralizing free radicals and prohibiting damage created by them (6). The role of fruit's antioxidants in providing protection against several diseases and postpone aging progression have been shown previously. The fruits that contain antioxidants have health increasing effect by deactivating free oxygen radicals (7). Many compositions such as

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polyphenols (8), flavonoids (9) and ascorbic acid (10) help antioxidative confidants.

The genus Citrus of the family Rutaceae consist of several important fruits such as limes, lemons, oranges, mandarins, grapefruits, and sour orange. Citrus sinensis one of the main horticultural products, with global agricultural output of beyond 80 million tons per year (11). Citrus fruit is a well provided source of antioxidant compounds, such as flavonoids, phenolic compounds and ascorbic acid; all of these have important roles in decreasing risk of cancer. Nutrient intake of citrus fruit juice has been related to health benefit (12-14).

Past researches have demonstrated that the quantity of flavonoids like quercetin could prohibit cancer progression. Some investigations indicated the great impact of quercetin in the debarment of atherosclerosis, sinusitis, asthma, hives, and inflammation disorders (18).

So it is important to estimate the content of antioxidant compounds such as ascorbic acid, total phenol and total flavonoids in commercial orange juices. Therefore, the main purpose of this study was to measure the levels of ascorbic acid, total phenolic and flavonoids in commercial orange juices and compares the concentration of these antioxidants among different brands.

2. Materials and Methods

2.1. Chemicals

All solvents, reagents and standards were purchased from Sigma (St. Louis, MO) and Merck (Darmstadt, Germany). A UV visible Cintra 40 double-beam spectrophotometer equipped with a 1.0 cm path length cell which connected to IBM compatible Pentium 100 computers was used.

2.2. Sample preparation

One hundred commercial orange juice samples were purchased from super markets in Tehran, Iran. The samples were from four different brands. Samples were centrifuged at 6000 rpm for 15 min at 20°C and after that filtered with whatman No. 4 filter paper. The samples were analyzed before expiry dates and stored in refrigerator. This research was conducted during the spring and summer months.

2.3. Measurement of total phenolic

Total phenols were measured colorimetrically by the Folin-Ciocalteu method (25) with slight modifications. About 300 µL of prepared samples was mixed with 1.5 mL of Folin-Ciocalteu reagent (previously diluted 10 fold with distilled water) and allowed to stand at room temperature for 5 min. Then 3 mL sodium bicarbonate solution (60 g/L) was added to the mixture and was incubated for 90 min at room. The level of absorbance was determined by a UV-Visible spectrophotometer at 725 nm. The total phenolic content was quantified by the calibration curve obtained from measuring the absorbance of 5 different concentrations (25, 50, 75, 100, 125 µg/mL) of gallic acid (GA) standard. The results were calculated as gallic acid equivalent (GAE) per 100 mL of initial samples and reported as mean value ± SD.

2.4. Measurement of total flavonoid

Total flavonoid content was determined by the aluminum chloride (AlCl₃.6H₂O) colorimetric assay (19). About 15 mL of samples or standard solution of quercetin (5, 10, 20, 30, 40 and 50 mg/L) was added to 25 mL volumetric flask containing 1 mL of 2 g/100 mL aluminum chloride in 5% acetic acid in methanol and the flask reached the volume 25 mL by this 5% acetic acid in methanol. After 30 min of incubation at room temperature, the samples absorbance was determined at 415 nm against solution of 5% acetic acid in methanol. Results were expressed as mg quercetin equivalents per 100 mL of initial samples and reported as mean value ± SD.

2.5. Measurement of ascorbic acid

Ascorbic acid content was determined by dinitrophenyl hydrazine method that established by Mc Comick and Wright (26). According to this method 80 µL of DTC (2, 4-dinitrophenylhydrazine thiourea copper (II) sulfate solution) was added to 75 µL of prepared samples. Then the solutions were put in a water bath for 3 h at 37°C. After adding 600 µL of 65% sulfuric acid to them, they were shaken to exit the gas. Then the samples were allowed to stand at room temperature for 30 min and the absorbance was measured at 520 nm with a spectrophotometer. The standard calibration curve was obtained from

absorbance of 400 μL of 7 different concentration (2.5, 5, 7.5, 10, 15, 20, 25 $\mu\text{g}/\text{mL}$) of ascorbic acid. The result was calculated based on a standard curve of vitamin C and reported as mean value \pm SD.

3. Results

3.1. Total Phenol

The total poly phenolic contents of 100 commercial orange juice was determined and equivalence by gallic acid as standard. The results were presented as the means \pm SD of total phenol in table 1. Calibration formula that obtained from 5 different concentrations of gallic acid was $y = 0.0101x + 0.0582$ ($r^2 = 0.9768$).

Table 1. The total phenolic contents in 100 samples from four different brands

brand	Number of samples	Mean Phenol ($\mu\text{g}/\text{mL}$)	SD	Min ($\mu\text{g}/\text{mL}$)	Max ($\mu\text{g}/\text{mL}$)
1	30	95.2989	10.74396	77.781	114.207
2	25	32.4455	2.46217	28.393	36.962
3	25	48.7296	3.63233	40.701	54.255
4	20	78.5513	10.21763	56.016	92.473
total	100	64.5937	26.61042	28.393	114.207

3.2. Total Flavonoid

The total flavonoid results were reported as the means \pm SD in table 2. Calibration formula that obtained from 6 different concentrations of quercetin was $y = 0.0312x + 0.0261$ ($R^2 = 0.9911$).

Table 2. The total flavonoid contents in 100 samples from four different brands

brand	Number of samples	Mean Flavonoid ($\mu\text{g}/\text{mL}$)	SD	Min ($\mu\text{g}/\text{mL}$)	Max ($\mu\text{g}/\text{mL}$)
1	30	28.5909	2.14989	23.38	32.62
2	25	14.5798	1.15965	12.53	16.84
3	25	26.6263	2.80911	20.21	30.11
4	20	24.9259	3.64748	14.20	30.76
total	100	23.8640	6.06948	12.53	32.62

3.3. Ascorbic Acid

The mean ascorbic acid concentration in commercial orange juices was shown in table 3. Calibration formula that obtained from 7 different concentrations of quercetin was $y = 0.0469x + 0.0158$ ($R^2 = 0.9901$).

Table 3. The total ascorbic acid contents in 100 samples from four different brands

brand	Number of samples	Mean Ascorbic acid ($\mu\text{g}/\text{mL}$)	SD	Min ($\mu\text{g}/\text{mL}$)	Max ($\mu\text{g}/\text{mL}$)
1	30	68.9075	9.31631	49.90	85.20
2	25	72.8479	10.03701	48.67	91.36
3	25	47.6290	6.14936	29.95	60.67
4	20	75.3719	11.98878	53.32	93.08
total	100	65.8658	14.28122	29.95	93.08

4. Discussion

Dainty citrus fruits have the health profit due to antioxidant properties (15). Ascorbic acid, also identified as vitamin C, is a very great antioxidant in fruits that defends fruits from oxidative stress (7). Ascorbic acid has various biological activities, such as synthesis of neurotransmitters, hormones and collagen (16). Ascorbic acid has been proposed an antioxidant effect that under certain status can protect against oxidative conclude DNA damage (17). It has an appointive role in scurvy prevention and is a potent antioxidant with water solubility (18). Flavonoid compounds in fruits are naturally presenting antioxidants (19,20). They have been displayed to prohibit tumorigenesis and metastasis (21,22), and many are accepted to have anti-inflammatory, antibacterial and antifungal capabilities (23). These effects are mainly attributed to their antioxidant activity (24).

The results of our study showed the concentration of total phenol varied from 28.39 to 114.20 mg GAE/L. The range of total flavonoid in the present report was reported between 12.53 to 32.62 mg QE/L. Significant difference was observed in the level of total flavonoids among four brands using Anova test ($p < 0.05$). But no statistically significant difference between brands 2 and 3 has been observed. The ascorbic acid level in commercial samples was in the range of 29.95-93.08 mg/L. A statistically comparison of the concentration of all parameters with Anova test indicates significant difference between them ($p < 0.05$). As it can be seen there is a significant difference between the reported results in the present work with previous. This difference may be caused by unrefreshing of four brands in our study as the samples were collected from the supermarkets. Also the orange juices in previous study are 100% pure whereas our samples had only 20% orange juice. The difference between orange juice content of samples justify the different result of our and other study.

Ammari and his colleagues in 2015 determined the concentration of total flavonoids in two commercial juices labeled as containing 45% orange juices 88.39 ± 0.43 mg QE/L (27). Klimczak and his partners in 2007 measured the level of total polyphenols by Folin-Ciocalteu method. The result was 684.2 ± 1.0 and 634.6 ± 0.9 mg of caffeic acid equivalent per liter (CAE/L) for two different brands (25).

Kabasakalis and his colleagues in 2000 determined ascorbic acid content of commercial 100% orange juice. The result was 42.4 mg/100 mL in long-life commercial orange juices and 42.7 mg/100 mL in short-life and the decrease of ascorbic acid after storage for 4 months at room temperature in closed containers was reported as 28.9% (28). A study was carried out in 2009 by Pisoschi to measure ascorbic acid content in juice samples by using the cyclic voltammetry method. The reported amount of ascorbic acid was between 146.08 and 293.92 mg/L (29). Gardner and his partners in 2000 reported the ascorbic acid concentration level of pure commercial orange juice 1233 ± 36 mg/L. They also reported total phenolic content of their samples as 755 ± 18 mg GAE/L (30). Rekha and his colleagues in 2012 in India investigated the ascorbic acid and total phenolic content of fresh juice of ripe and unripe *Citrus sinensis*. The results showed that the total phenol level was 820 mg GAE/L for ripe orange and 960 mg GAE/L for unripe. The determined ascorbic acid content was 17.4 g/100 mL in ripe orange juice and 19.04 g/100 mL in unripe orange juice (31).

5. Conclusion

The necessity of antioxidants consumption is growing to prevent the effects of free radicals. Several researches demonstrated the effects of fruits and vegetables antioxidant, and say that fruits are good source of polyphenols, total phenols and vitamin C. Safety assessments of different brands of orange juice are assessed with factors like the acidity, formalin index, dry content, pH and Brix. Therefore, in the current research the total phenols, flavonoids and vitamin C levels in four commercial orange juice brands were measured and compared. The Institute of standards and Industrial Research of Iran just state the maximum additive concentrations of ascorbic acid as 400 mg/kg. Comparison between the ascorbic acid content which was determined in current study and the maximum additive concentrations demonstrate that four brands of current study had less ascorbic acid. International standard organization doesn't set any sufficient amount for the level of mentioned antioxidant in our article. So it's suggested that Codex determines a minimum concentration of ascorbic acid, total phenol, total flavonoid and other antioxidants for fruit juice

including 100% pure juice, nectar and fruit juice without gas.

Conflict of interest

The authors declare that there is no conflict of interest.

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