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BIOACTIVE COMPOUNDS, ANTIOXIDANT AND PHYSICO-CHEMICAL PROPERTIES OF JUICE FROM LEMON, MANDARIN AND ORANGE FRUITS CULTIVATED IN SAUDI ARABIA

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Abstract

Juice prepared from lemon (Eureka), mandarin (Kinnow) and orange (Orlando) fruits cultivated in Saudi Arabia were analyzed for different physico-chemical properties, total phenolics, ascorbic acid and antiradical activities. Juices from lemon, mandarin and orange 8.97, 16.1 and 11.78% total soluble solids; 5.73, 1.37 and 1.69% titrable acidity; 79.21, 91.18 and 107.37 mg GAE/100mL total phenolics; 31.24, 53.15 and 53.24 mg/100mL ascorbic acid and 48.3, 59.19 and 61.35% DPPH radical scavenging activities, respectively. The results show that juice from locally grown citrus fruits is of good quality and a valuable source of health promoting constituents.

Introduction

Citrus fruit is very popular in many parts of the world due to its distinctive flavor, taste, and aroma as well as multiple health benefits associated with it. The consumption of citrus fruits or their products is believed to have beneficial effects against different diseases, the main reason being the presence of important bioactive compounds (Pellegrini *et al*., 2003; Peterson *et al*., 2006).It is now widely known that vitamin C (ascorbic acid) and carotenoids are found in abundance in citrus fruits (Dhuique-Mayer *et al*., 2005), which play an important roles primarily in causing resistance against many diseases. Recently, the focus has shifted to phenolic compounds derivedfrom different fruits including citrus. For example, some studies have suggested that in addition to vitamin C and carotenoids, phenolic compounds play an integral role in total antioxidant capacity of citrus fruits (Gorinstein *et al*., 2004). The major phenolic compounds detected in different citrus fruits are categorized as flavonoids and phenolic acids (Balasundram *et al*., 2006). Lemon (*Citrus limon*) is a small tree and originated probably from Asia. The fruit of lemon tree is green to yellow in color, and it is used in making various foods, due to its distinctive flavor and ability to enhance spicy flavor of certain foods. The lemon juice is a major product of lemon fruit obtained on commercial scale. Orange or sweet orange, belongs to citrus species *Citrus sinensis* from Rutaceae family. Mandarin (*Citrus reticulata*) on the other hand is produced on small trees (Anon., 2013a).

Different studies have reported the health benefits of citrus fruits in terms of their antioxidant capacity. It was suggested in most cases that the total antioxidant power of such fruits was a combined ability of vitamin C and phenolics to impart different antioxidant characteristics, such as chelation of ions, ferric reducing ability, etc. However, there is more need to specify which type of compound from citrus fruit imparts a certain antioxidant function (Gardner *et al*., 2000; Rapisarda *et al*., 1999; Yoo *et al*., 2004). The annual production of citrus fruits in Saudi Arabia was 135000 and 146845tonnes in 2010 and 2011 (Anon., 2013b), respectively, which indicates that the production of different citrus species is consistently increasing in the Kingdom. However, research work on appraising the quality of citrus fruit produced in Saudi Arabia, particularly with reference to bioactive compounds from citrus products and their health benefits has not been properly carried out.

As there is an increase in the production of citrus fruits in Saudi Arabia, so the demand for processed citrus products such as juice is also increasing in recent years. However, there is still considerable need to explore the antioxidant and bioactive potential of locally grown citrus fruits. The aim of carrying out this study was to explore the antioxidant potential and quantify the bioactive compounds, namely vitamin C and phenolics in the juice from lemon, orange and mandarin grown in Saudi Arabia, in addition to analyzing it for different quality characteristics.

Materials and Methods

Three main citrus fruits cultivated in Riyadh, Saudi Arabia were collected from a local farm whereby the farmer produced world famous cultivars of citrus in the local environment. Lemon cultivar was “Eureka” while those of orange and mandarin were “Orlando” and “Kinnow”, respectively. The selected fruits were at mature stage; they were cut into halves and juice was squeezed with hands till all juice recovered. The juice was passed through a cheese cloth, juice yields were calculated and samples were taken for further physico-chemical analysis.

Total solids, pH and acidity measurement: Citrus juice was analyzed for total soluble solids (TSS) using a Digital Refractometer (Model: Abbe Mark II, Cambridge Instrument, INC. Baffalo, NY, USA). The machine was standardized using purified water before taking readings. Titrable acidity (TA) was determined as percentage using a previously described method (AL-Juhaimi *et al*., 2012). The pH was measured using a pH meter (Model: pH meter 240, Corning Scientific Products, NY, USA).

Analysis for total phenolic compounds: The total phenolic compounds were evaluated using a modified method that involved Folin Ciocalteure agent (FCR) (Ghafoor *et al*., 2012). A juice sample (200µL) was diluted to the extent so as to obtain a desired range of absorbance value within a standard curve prepared by using varying concentrations of gallic acid as standard. The juice dilutions or standard solution preparations were done using deionized water. The sample containing FCR was diluted furtherto obtain a final volume of 4.6 mL followed by thorough mixing. The sample was kept at room temperature for 10 min and 1 mL of 20% Na_2CO_3 solution was added, followed by vortex mixing and incubation at room temperature for another 2 h. The absorbance was read at 765 nm on a spectrophotometer (Ultrospec II 4050; LKB Biochrom, Cambridge, England) against a blank containing all reagents without the samples or the gallic acid at the same conditions.Total phenolic compounds were quantified as milligram gallic acid equivalent per 100 mL of juice or mg GAE/100mL.

Determination of ascorbic acid contents: The quantification of ascorbic acid contents of citrus juice was done using a modified phosphomolybdenum complex method which is generally used to express antioxidants as equivalents of ascorbic acid contents (Ghafoor *et al*., 2012). Breifly, 0.4mL of diluted juice sample (100µL of citrus juice diluted using 1mL of methanol) was combined with 4 mL of the reagent solution that contained sulphuric aicd, sodium phosphate and ammonium molybdate solutions of 4mM, 2mM and 0.6M molarities, respectively. A blank solution was also prepared which consisted of 4 mL of the reagent solution and 1mL of methanol. The test tubes carrying the thoroughly mixed samples prepared as explained above were tightly caped and placed in a water bath at 95 C for 90 min. The samples were cooled to room temperature using tap water and the absorbance read at 695 nm against a blank. The ascorbic acid was used as a standard to obtain a calibration curve and its corresponding contents in the citrus juice samples were expressed as mg of ascorbic acid per 100 mL (mg/100 mL).

DPPH radical scavenging activity: The free radical activity of juice from lemon, mandarin and orange fruits was evaluated using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) (Ghafoor *et al*., 2010). One mL of the juice sample (100µL/mL methanol) was dissolved in 2mL of DPPH solution (10 mg DPPH dissolved in 1 L methanol). The samples were thoroughly mixed using a vortex mixer and left to incubate at room temperature for the desired de-coloration of DPPH solution in 5 min. The absorbance was measured at 517 nm. From the practical point of view, the lower absorbance of a juice sample reflected that it had a better ability to scavenge DPPH radicals. The results were expressed as percentage of DPPH radicals scavenged by 1 mL of citrus juice.

Statistical analysis: The analytical measurements were carried out in triplicates and the data presented as means ±SD. Statistical analysis was performed using the Sigma Plot (Version 10, Systat Software Inc., Chicago, IL, USA). Analysis of variance of all data sets was worked out and the mean values were considered significantly different at $p<0.05$.

Results and discussion

Yield, total soluble solids (TSS), pH and titrable acidity (TA) of citrus juice: Different characteristics, such as juice yield, TSS, TA,and TSS/TA ratio as shown in Table 1, were chosen to characterize different juices in terms of overall quality. The data showed a considerable variation in juice from different citrus fruits. Mandarin fruit resulted in highest juice yield (51.82 %) of all fruits. The lowest juice yield (42.83%) was from lemon, the same juice had the lowest total soluble solids and acid, but the highest acidity. TSS/TA ratio, which is among important quality attributes of juices, was higher in the case of mandarin juice (11.75), however, the lemon juice had the lowest (1.57) values. From the above data, significant differences among 3 types of citrus fruits were observed in terms of different physico-chemical characteristics. Of all citrus types, the mandarin juice showed best quality characteristics in terms of the earlier-mentioned parameters. These results were also consistent with some other reports (Xu *et al*., 2008). In terms of acidity, the orange juice from Orlando cultivar grown in Saudi Arabia was observed to be less acidic reflecting its considerable sweetness.

Total phenolic compounds and ascorbic acid contents: The total phenolic compounds in three different citrus juices evaluated by the Folin Ciocalteu method are presented in Fig. 1. Orange juice from Orlando type oranges was the best in terms of phenolic compounds being maximum (107.37 mg GAE/100 mL), followed by mandarin (Kinnow) juice (91.18 mg GAE/100 mL). The lemon (Eureka) had the lowest amount of these bioactives (79.21 mg GAE/100mL of juice).

Table 1. Total soluble solids, pH and titrable acidity and yield of freshly extracted juice from Saudi citrus fruits.

Common name	Species name	Juice yield (%)	TSS (%)[a]	pH	TA (%)[b]	TSS/TA ratio
Lemon	*Citrus limon* cv. Eureka	42.83 ± 2.82	8.97 ± 0.86	2.32 ± 0.01	5.73 ± 0.03	1.57
Mandarin	*Citrus reticulata* cv. Kinnow	51.82 ± 1.95	16.1 ± 0.69	3.18 ± 0.03	1.37 ± 0.04	11.75
Orange	*Citrus sinensis*cv. Orlando	45.26 ± 1.76	11.78 ± 1.05	3.41 ± 0.05	1.69 ± 0.06	6.97

[a]Total soluble solids

[b]Titrable acidity

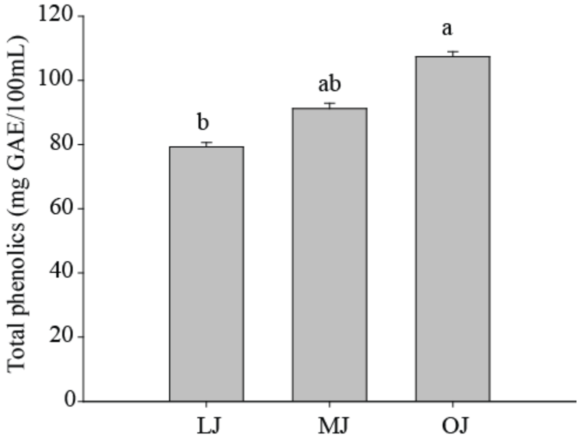

Fig. 1. Total phenolic compounds in freshly extracted juices from lemon (LJ), mandarin (MJ) and orange (OJ). Bars with different small letter are significantly different (p<0.05).

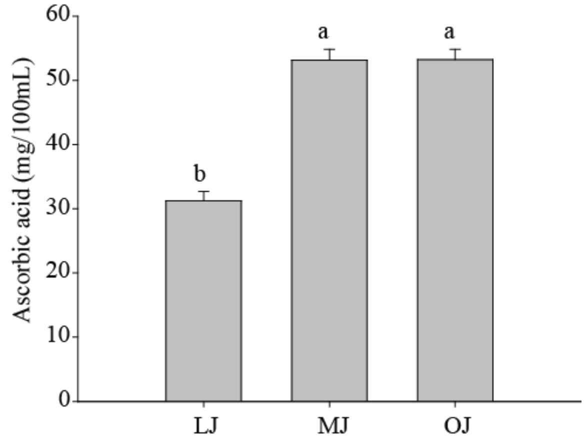

Fig. 2. Ascorbic acid contents of freshly extracted juices from lemon (LJ), mandarin (MJ) and orange (OJ). Bars with different small letter are significantly different (p<0.05).

The phenolic compounds have various biologically important functions such as radical scavenging, antioxidants and other health promoting properties (Ghafoor *et al*., 2011a, AL-Juhaimi & Ghafoor, 2011). Prolonged consumption of phenolic compounds is associated with prevention and reduction of obesity and effects on metabolic pathways such as secretion of a dipokine and oxidative stress (Decorde *et al*., 2009). They can also be recommended as natural food additives and preferred over synthetic antioxidants such as butylatedhydroxyanisole and butylatedhydroxytoluene (Ghafoor *et al*., 2011a). The multiple mechanisms of their anti-oxidative activities are expressed in their abilities of radical scavenging, metal chelation, and synergism with other antioxidants (Lim *et al*., 2011). The presence of substantial amounts of these compounds in juice obtained from locally cultivated citrus cultivars make them important for their utilization in processing different products. These results were in agreement with some previous reports concerning citrus fruits (Caro *et al*., 2004; Xu *et al*., 2008).

The ascorbic acid contents of mandarin and orange juice (Fig. 2) did not differ significantly, but those of lemon juice were markedly lower (31.24 mg/100mL) which were in agreement with the findings of Xu *et al*., (2008) in which a similar trend in the amounts of ascorbic acid was observed in the juices of lemon, orange and mandarin. However, our results differ from that report because therein, the quantity of ascorbic acid of mandarin juice was shown to be significantly higher than that of orange juice. The reason may be analytical differences, geological effects and the difference in cultivars.

Antiradical activity of lemon, mandarin and orange juice: The antiradical activity of 3 kinds of juices as observed by the DPPH radical scavenging method is presented in Fig. 3. The DPPH radical scavenging and other types of methods for detecting antioxidant capacity are often used for plant and food extracts (Ghafoor *et al*., 2011b; Lim *et al*., 2010). The orange juice had highest (61.35%) free radical scavenging activity followed by mandarin (59.19%) and lemon juice (51.31%) which was similar to the trend observed in phenolic acid contents of these juices. However, in order to compare the strength of these activities in terms of phenolic compounds of a respective juice we also calculated the radical scavenging activity of each juice as a function of phenolic compounds and it was observed that mandarin juice phenolics seemed to have better ability for scavenging free radicals, i.e. in terms of their proportion they scavenged relatively more free radicals than did by the orange juice. This gives an indication that mandarin juice has better quality due to possessing better biologically available phenolics among these three types of fruits. However, we cannot attribute this ability to only phenolic compounds because ascorbic acid in citrus juice is also a main contributor of antioxidant capacity. In previous reports (Arena *et al*., 2001; Yoo *et al*., 2004) it was suggested that ascorbic acid played more integral role than phenolic compounds in formulating the total antioxidant power of juices from citrus. On the other hand, some studies revealed that phenolcis dominated vitamin C in establishing total antioxidant capacity of juice (Sun *et al*., 2002). It is possible that different factors may have contributed to these differential findings such as the type of citrus cultivar, stage of maturity and the methodology selected for analyzing antioxidants. In general it was observed that orange juice grown in Saudi Arabia had higher antioxidant power than mandarin or lemon juices, the reason might be higher phenolic contents in this juice.

Conclusions

The citrus fruits grown in Saudi Arabia were evaluated for the quality of juice, total phenolics, ascorbic acid and antiradical activities. Juice from lemon (Eureka), mandarin (Kinnow) and orange (Orlando) had 8.97, 16.1 and 11.78% total soluble solids; 5.73, 1.37 and 1.69% titrable acidity; 79.21, 91.18 and 107.37 mg GAE/100mL total phenolics; 31.24, 53.15 and 53.24 mg/100mL ascorbic acid and 48.3, 59.19 and 61.35% DPPH radical scavenging activities, respectively. Our results reveal that juice from locally grown citrus fruits is of good quality and a valuable source of health promoting constituents, hence it can be effectively used for production of different food products. The results also encourage the cultivation of different kinds of citrus and other fruits in the local environment of the Kingdom.

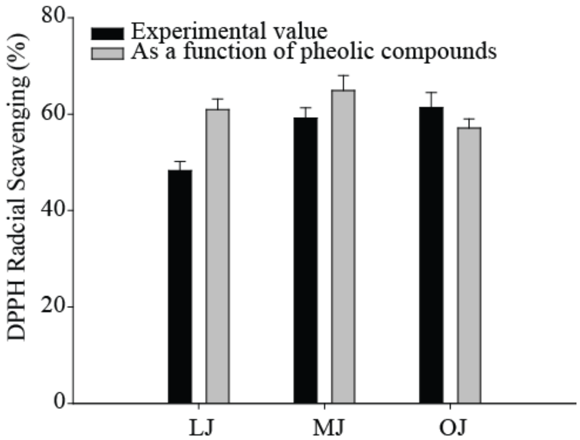

Fig. 3. Anti-DPPH radical activities (experimental and as function of total phenolics) of freshly extracted juices from lemon (LJ), mandarin (MJ) and orange (OJ) cultivated in Saudi Arabia.

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