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CITRUS FRUITS BY-PRODUCTS AS SOURCES OF BIOACTIVE COMPOUNDS WITH ANTIOXIDANT POTENTIAL

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Abstract

Peel and pulp from Orlando orange, Kinnow mandarin and Eureka lemon fruits were evaluated for phenolic compounds, ascorbic acid contents and free radical scavenging activities. Pulp from orange, mandarin and lemon contained 123.02, 104.98 and 98.38 mg GAE/100 g total phenolics; 61.38, 38.52 and 57.63 mg/100g ascorbic acid and 69.31, 62.82 and 59.60 % DPPH radical scavenging activities, respectively. Peel from orange, mandarin and lemon contained 178.90, 169.54 and 61.22 mg GAE/100 g total phenolics; 62.45, 54.87 and 25.68 mg/100g ascorbic acid and 67.58, 68.57 and 46.98% DPPH radical scavenging activities, respectively. The data reveals that these citrus by-products are good sources of bioactive compounds and be considered as antioxidant constituents for developing functional foods.

Introduction

Citrus fruit is popular due to its characteristic flavor, taste, aroma and multiple health benefits. Citrus fruits and products containing citrus are known for different health benefits and prevention of diseases in human. The existence of important bioactive compounds in citrus fruits is the major reason for different biological properties. Ascorbic acid (vitamin C) and carotenoids, the important contributors for different health properties of citrus fruits, are present in abundant quantities which is now a well established fact (Al-Juhaimi & Ghafoor, 2013), that produce resistance against different diseases. There is also increasing trend towards research in phenolic compounds present in citrus. The presence of phenolic compounds in citrus fruits is also reported and that they also contribute to the antioxidant properties similar to vitamin C and carotenoids type compounds (AL-Juhaimi & Ghafoor, 2013). Flavonoids and phenolic acids determine a major part of bioactive compounds for citrus fruits (Astell *et al.,* 2013). *Citrus limon* is the botanical name of lemon plant, which is a short tree that has its origin perhaps from Asia. The color of lemon fruit varies from green to yellow depending on the maturity and type of cultivar. Orange or sweet oranges referred to as *Citrus sinensis* botanically and belongs to *Rutaceae* family. Mandarin (*Citrus reticulata*) is another famous citrus fruit that has bright orange color (AL-Juhaimi & Ghafoor, 2013).

The citrus fruit production in Saudi Arabia is increasing and 135000 and 146845 tons of it were produced in 2010 and 2011, respectively. Processing of citrus fruits into different products or their consumption as such produce by-products such as peel, seed, and pulp which are usually wasted. Hence there is a need to evaluate the bioactive potential of by-products from citrus fruit produced in Saudi Arabia, so as to find out the possibilities of their use in different food formulations or manufacture of health promoting products or functional foods. These byproducts can be used in animal feed as molasses, and for fibre (pectin) and green energy production (Li *et al.*, 2006). The bioactive compounds from citrus byproducts such as peel and pulp and other fruit wastes can be evaluated as alternative to synthetic food additives which are associated with negative effects on human health (Ignat *et al.*, 2011).

The aims of this study were evaluation of antiradical activities and quantification of bioactives including ascorbic acid and phenolic compounds in peel and pulp from orange, lemon and mandarin fruits grown in Saudi Arabia.

Materials and Methods

Three different locally grown citrus fruits were obtained from a farm near Riyadh, Saudi Arabia. The cultivars were identified as Eureka, Orlando and Kinnow for lemon, orange and mandarin, respectively. Fully mature and ripe fruits were selected and cut into halves to squeeze juice. The juice was filtered using a cheese cloth, the pulp part that remained on the cheese cloth was collected and freeze dried. Peels were also removed manually from fruits and then dried in freeze dryer. After drying both pulp and peel samples were ground to a powder form, using a blender, for further analyses. Samples for analysis were prepared by using 50 mL ethanol (80%) as solvent and extraction from 1 g peel or pulp sample was carried out at 70°C in a shaking water bath at medium speed for 3 h. Followed extraction the samples were filtered and volume of the extract fixed using ethanol (80%) and taken for further analyses.

Determination of total phenolics: The total phenolic compounds were evaluated using a modified Folin Ciocalteu Reagent (FCR) method (Ghafoor & Choi, 2009). An extract sample (as obtained by processes explained in previous section) was diluted using distilled water and gallic acid (standard compound) solutions of varying concentrations were used for the calibration curve. One mL of FCR was added to 200 µL of sample or standard solution and mixed thoroughly. After 10 min incubation at room temperature 1mL of 20% Na_2CO_3 solution was added, followed by thorough mixing. The solutions were incubated further at room temperature for 2 h and absorbance values were recorded using a spectrophotometer (UltrospecII; Biochrom-LKB, Cambridge, England) using a blank for auto zero at 765 nm. The blank carried all reagents as previously mentioned but was without either sample or standard solution. Total phenolics were expressed as milligrams of gallic acid equivalent per 100 gram or mg GAE/100 g of dried peel or pulp from individual citrus fruits.

Quantification of ascorbic acid: The ascorbic acid contents were quantified using a modified phosphomolybdenum complex method that can also be used to determined total antioxidants as equivalents of ascorbic acids in a plant extracts (Ghafoor *et al*., 2012). In brief, 0.4mL sample (diluted peel or pulp extracts using methanol) was mixed with 4 mL reagent (mixture of 4mM NaH_2PO_4, 2mM H_2SO_4, and 0.6M $(NH_4)_2MoO_4$ solutions). 4 mL reagent and 1mL methanol were joined to get blank. The tubes were tightly caped and sealed using paraffin wax and put at 95°C (in a water bath) for 90 min. After this treatment samples were immediately cooled to ambient temperature under running water. The values of absorbance were recorded at 695 nm against a blank. The pure ascorbic acid standard was used to obtain a calibration curve and its comparative contents in the peel and pulp extracts were presented as milligrams of ascorbic acid per 100 gram (mg/ 100 g) of either of the dried pulp or peel.

Free radical scavenging ability: The values for radical scavenging activities of peel and pulp from three citrus fruits were obtained using diphenyl picrylhydrazyl (DPPH) method (Ghafoor, 2014). Extract sample (1 mL) was mixed with of DPPH solution 2mL which was obtained by mixing 10 mg DPPH in methanol and total volume made to 1 L using methanol. These mixtures were vortexed for thorough mixing and incubation at 25°C or room temperature was carried out for 5 min so that the desired color, after free radicals reaction with sample solution, was developed. The absorbance values were recorded at 517 nm. The lower absorbance value of the sample gave an indication that it effectively scavnenged free radical of DPPH solution and hence the dark color of this solution changed to lighter one. The results presented DPPH radicals (%) eradicated by 1 mL peel or pulp extract.

Statistical analysis: All the analysis for each parameter were done in triplicates (n = 3) and results presented as means ±SD. Statistical analysis was accomplished using Sigma Plot (Systat Inc., Chicago, IL, USA). Analysis of variance was calculated for the data and values were considered as significantly different at $p<0.05$.

Results and Discussion

Total phenolics and ascorbic acid contents: The total phenolic compounds in peel and pulp of three different citrus fruits were evaluated using Folin Ciocalteu reagent and results shown in Fig. 1. The maximum phenolic compounds (178.90 mg GAE/100 g) were obtained from Orlando orange peel followed by Kinnow mandarin peel (169.54 mg GAE/100 g). Peel from Eureka lemon peel demonstrated lowest quantity of phenolics (61.23 mg GAE/100 g) among different samples analyzed. The pulp from orange, lemon and mandarin showed 123.02, 98.38 and 104.98 mg GAE/100 g of phenolic compounds, respectively.

The contents of ascorbic acid in these six different samples are shown in Fig. 2. Peels from orange, lemon and mandarin contained 62.45, 25.68 and 54.87 mg/100 g of ascorbic acid, respectively. The quantity of pulp ascorbic acid was comparable to those of peel of respective fruit. The pulp from orange, lemon and mandarin contained 61.38, 38.52 and 57.63 mg/100 g of ascorbic acid, respectively. In general the ascorbic acid contents of peel and pulp of orange was higher than those of mandarin and lemon. Lemon peel and pulp showed lower quantities of phenolic compounds and ascorbic acid than other two types of citrus fruits. A similar trend in these two types of bioactive compounds was also observed in juice samples of these three fruits in another study (Al Juhaimi & Ghafoor, 2013) where it was observed that lemon, mandarin and orange juices contained 79.21, 91.18 and 107.37 mg GAE/100mL total phenolics and 31.24, 53.15 and 53.24 mg/100mL ascorbic acid. The results of present study also suggest that the pulp and peel of these three citrus fruits are slightly better sources of phenolic compounds and ascorbic acid in terms of their quantities.

The bioactive compounds such as phenolic compounds are responsible for valuable antioxidant potential of extracts from different plant materials such as fruits, seeds, leaves, and stems and these are regarded as health beneficial constituents (Ghafoor *et al*., 2011a, AL-Juhaimi & Ghafoor, 2011; AL-Juhaimi *et al*., 2014). The use of phenolics is also reported for lowering and preventing obesity; effecting secretion of a dipokine and prevention of oxidative stress (Dalar *et al*., 2014). These phenolic compounds from natural sources are recommendable as natural food additives and they are considered more suitable for application in food products than butylatedhydroxyanisole and butylatedhydroxytoluene which are artificial compounds with antioxidant properties (Ghafoor *et al*., 2011a). There are various actions which sum up the antioxidant potential of these compounds are such actions include scavenging free radical, metal chelation, and synergism with other antioxidants (Lim *et al*., 2011). The occurrence of high amounts of bioactive compounds in peel and pulp from locally grown citrus fruits show the importance of these citrus by-products due to their nutraceutical and health potential for use in functional foods.

Radical scavenging activities of lemon, mandarin and orange peel and pulp: The radical scavenging activities of peel and pulp samples from different citrus fruits were observed by free radical scavenging method that involve use of DPPH and the results are presented in Fig. 3. This method along with various other methods is often used for detecting antioxidant capacity plant and food extracts (Ghafoor *et al*., 2011b; Lim *et al*., 2010). The method involve use of DPPH radicals solution in methanol which has a darker color that becomes lighter after reaction with antioxidants in natural extracts. The orange pulp (69.31%) and peel (67.58%) and mandarin peel (68.57%) showed higher free radical scavenging activities. The lowest DPPH scavenging activity (46.98) was shown by lemon peel. The results demonstrate that all these by-products have a good potential to scavenging free radicals.

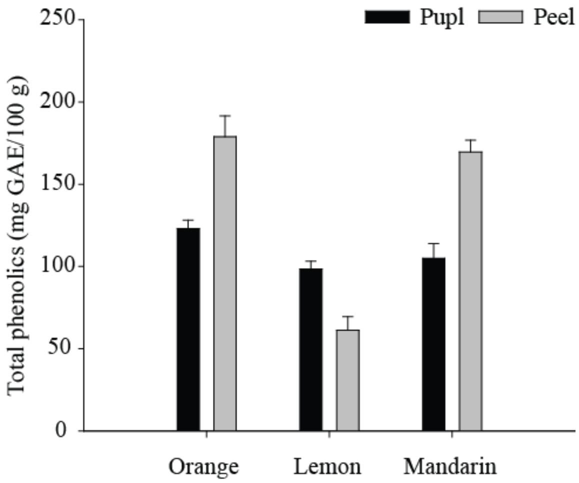

Fig. 1. Total phenolic compounds from peel and pulp of orange, lemon and mandarin fruits cultivated in Saudi Arabia.

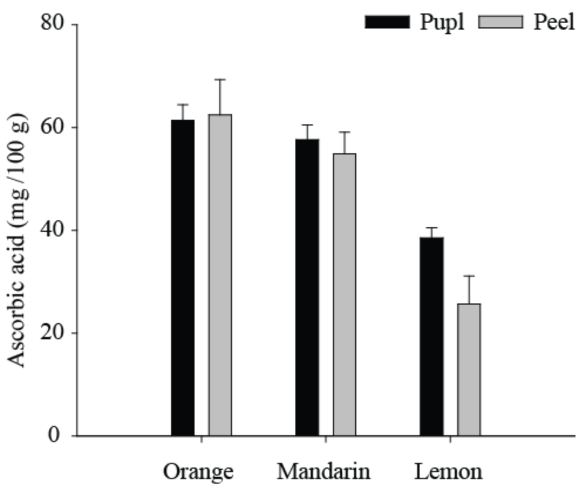

Fig. 2. Ascorbic acid contents from peel and pulp of orange, lemon and mandarin fruits cultivated in Saudi Arabia.

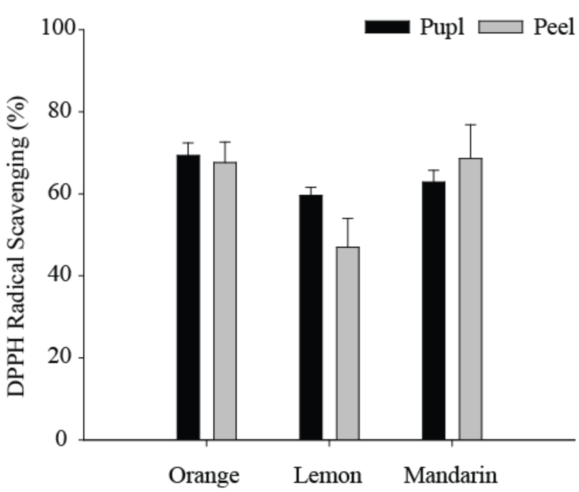

Fig. 3. DPPH radical scavenging activities of the extracts from peel and pulp of orange, lemon and mandarin fruits cultivated in Saudi Arabia.

In previous reports (Arena *et al*., 2001) it was observed that the vitamin C contents contributed more than phenolic compounds in establishing antioxidant power of citrus fruits. Citrus fruits are often cherished due to high contents of vitamin C contents which have multiple health benefits by preventing and curing certain diseases (Asikin *et al.*, 2014). Other studies also report that the total antioxidant power of plant extracts is governed to larger extent by phenolic compounds than ascorbic acid (Manganaris *et al*., 2013; Silva *et al.*, 2013). There is also possibility that various variable factors may have resulted in making such distinctive inferences and these factors may include maturity of plant or fruit and the analytical methods used in various studies for quantification of antioxidant power. However, it seems that the establishment of fact which type of bioactive compounds is more active for antioxidant action of plant extracts may be debatable. In general it was observed that peel and pulp from orange showed higher antioxidant activity which may be due to presence of more phenolic compounds in these by-products. Besides mandarin fruit peel and pulp were also good sources of bioactive compounds that also showed excellent *In vitro* radical scavenging properties.

Conclusion

The peel and pulp from three different citrus fruits were analyzed for phenolic compounds, ascorbic acid contents and radical scavenging properties. Pulp from orange, mandarin and lemon contained 123.02, 104.98 and 98.38 mg GAE/100 g total phenolics; 61.38, 38.52 and 57.63 mg/100g ascorbic acid and 69.31, 62.82 and 59.60 % antiradical activities against DPPH radicals, respectively. Peel from orange, mandarin and lemon 178.90, 169.54 and 61.22 mg GAE/100 g total phenolics; 62.45, 54.87 and 25.68 mg/100g ascorbic acid and 67.58, 68.57 and 46.98 % antiradical activities against DPPH radicals, respectively. These results reveal that peel and pulp from locally grown citrus fruits are valuable sources of health benefiting bioactive components, hence they can be considered for use in food products formulation with the objective of adding health benefits to foods such as increased antioxidant potential. The desert environment of Saudi Arabia is conducive for citrus production provided that appropriate agronomic practices are followed for obtaining high quality citrus fruit. Prudent use of by-products from plant sources can also be helpful for maximum utilization of natural foods and at the same time assist in environment protection.

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