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RIPENING- DEPENDENT CHANGES IN ANTIOXIDANT ACTIVITY AND PHENOLIC CONTENTS OF FRUIT RESIDUES OF KINNOW MANDARIN

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Abstract

Kinnow mandarin is one of the most commonly consumed fruits in India. The extracts from Kinnow peel, pulp, seed and juice for three different stages of ripening (IM, SM and CM) were evaluated for Total phenols (TP), Total flavonoids (TF) and antioxidant activity using DPPH free radical scavenging activity. The TP 9.37 ± 0.05 GAE /gm dw was highest for late stage juice, the highest value of TF was for late stage pulp 5.12 QE mg /gm dw whereas antioxidant activity 33.49 ± 0.075 mg AA/ gm dw was highest for early stage peel and pulp extracts. Our findings indicated that in Kinnow fruit residues some non phenolic components also contributed to the total antioxidant activity. This study probably provides first comprehensive data on TP, TF and antioxidant activity for the four Kinnow fruit residues especially with reference to different ripening stages. The present study demonstrates that the Kinnow peel, pulp, seed and juice are a potential source of antioxidants for food and pharmaceutical industries.

Key words : Kinnow mandarin, Phenols, DPPH, Antioxidant, fruit residues.

Introduction

Citrus are among the most popular fruits of the world. These act as natural antioxidants and provides protection against oxidative damage from free radicals and thus reduces the risk of disease occurrence (Zou *et al.,* 2016). Kinnow mandarin, a hybrid of two citrus cultivars – "King" x "Willow Leaf" is an important citrus crop in India (Ladaniya, 2008).

Phenols and Flavonoids are active biochemicals of fruits and vegetables. Several studies have reported that the fruit consumption is linked with a lower risk of chronic diseases (Beecher, 1999; Van't Veer *et al.,* 2000). Therefore regular consumption of fruits and vegetables is recommended for good health and also for reducing the risk of suffering due to disease (Singh *et al.,* 2016).

Along with other factors phenolic antioxidants seems to be responsible for these effects (Ruxton *et al.,* 2006; Saura-Calixto and Goñi, 2006).

Kinnow is an important crop and a natural source of antioxidants as it contains a number of significant biologically active compounds (Zhang *et al.,* 2018); therefore, there is an urgent need to investigate and evaluate the antioxidant potential of containments in different parts of Kinnow fruits. Present research was designed to determine and to compare the content of phenolic compounds and the antioxidant capacity of fruit residue extracts of Kinnow mandarin in this regard. Different ripening stages of the Kinnow fruit was taken into consideration.

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Materials and Methods

The fruits of Kinnow mandarin were collected from Kinnow mandarin orchard of Daal farm, Sriganganagar (Rajasthan) at three different ripening stages –Early, mid and late. The fruits were separated into peels, pulp and seeds. The juice was manually squeezed from the pulp. The methanolic extracts were prepared from dried and coarsely powdered plant materials.

Total phenols

Total phenols were determined by Follin Ciocalteau

Reagent method (McDonald *et al.*, 2001). An aliquot of each plant extract (0.5ml 1:10 mg/l) or gallic acid (GA: standard phenolic compound) was added with Follin Ciocalteu reagent (5ml 1:10 diluted with distilled water) and 4ml of 1M solution of Na_2CO_3. The mixture was allowed to stand for 30 minutes at room temperature and absorbance was measured at 710nm. Total phenols of extracts was expressed as mg Gallic acid equivalent (GAE)/gm fresh weight. All samples was analyzed in triplicates.

Total flavonoids

Total flavonoid were analyzed by Aluminum Chloride method (Chang *et al.*, 2002). Each plant extract (0.5 ml of 1:10 gm/lt) was mixed with 1.5 ml methanol, 0.1 ml of 10% $AlCl_3$, 0.1 ml of 1M potassium acetate and 2.8 ml distilled water. The mixture was allowed to stand for 30 minutes at room temperature and absorbance was measured at 415nm.Total flavonoid contents was expressed as mg quercitin equivalents (QE)/1gm fresh mass. Samples were analyzed in triplicates.

DPPH- free radical scavenging activity

(DPPH) was used for determination of free radical scavenging activity of the extracts (Koleva *et al.*, 2002). Different concentrations of each extract was mixed with methanolic solution of DPPH (0.004%). The mixture was allowed to stand for 15 minutes. The scavenging of free radicals by extract was evaluated spectrophotometrically at 517nm against the absorbance of DPPH radicals. The percentage discoloration was calculated by following formula.

DPPH radical scavenging activity (%)

$$= \left[AC_{517} - \frac{AE_{517}}{AC_{517}} \right] \times 100$$

Where AC_{517} is absorbance of a DPPH solution without extract, AE_{517} is the absorbance of the tested plant extract with DPPH.

Results and Discussion

Total phenol

Methanol was used as a solvent for extracting bioactive compounds as it is an effective solvent for antioxidant extraction (Sidhuraju and Becker, 2003). The results in Table 1 show the presence of phenolic compounds among Kinnow fruit residues with varying proportions. The range of TP in present investigation is from 0.37 mg GAE/g dw (mid stage juice) to 9.37 mg GAE/g dw (late stage juice). In early and mid stage, pulp has the highest phenol values while in late stage maximum phenol is in juice The average of the phenolic content in commercially ripened stage of Kinnow fruit when ordered from high to low was as follows: juice (9.37 mg GAE/g dw) > pulp (6.49 mg GAE/g dw) > peel (5.49 mg GAE/g dw)> seed (1.5 mg GAE/g dw). The trend of TP concentration in this stage is much lower (except seed) than those of peel (27.18 mg GAE/g dw) > juice (24.98 mg GAE/g dw) > pulp (12.33 mg GAE/g dw)>seed (1.71 mg GAE/g dw) reported by Zhang et al (2018) and those reported by Babber et al (2011), but was higher than those (0.6 mg GAE/g dw) published by Chen et al (2010).The reasons for these differences in TP may be related to different geographical origin, genetic background, harvesting time, cultivar and drying and

Table 1: Total phenols and Ascorbic acid equivalent antioxidant capacity of methanolic extracts obtained from fruit residues of Kinnow mandarin.

Fruit Residue	Fruit ripening stage	Phenols GAE mg/gm dwt. ± SD	Flavonoids QE mg/gm dwt. ± SD	Antioxidant capacity mg/gm dwt. ± SD
Peel	Early Stage	3.66 ± 0.075	0.25 ± 0.125	33.49 ± 0.025
	Mid Stage	2.99 ± 0.125	0.625 ± 0.05	30.5 ± 0.05
	Late Stage	5.49 ± 0.125	4.5 ± 0.011	29.74 ± 0.075
Pulp	Early Stage	6.74 ± 0.025	2 ± 0.05	33.25 ± 0.011
	Mid Stage	5.24 ± 0.025	3.99 ± 0.025	29.74 ± 0.075
	Late Stage	6.49 ± 0.025	5.12 ± 0.05	29.49 ± 0.025
Seed	Early Stage	No Seed Formation	No Seed Formation	No Seed Formation
	Mid Stage	0.87 ± 0.025	1.5 ± 0.011	26.24 ± 0.075
	Late Stage	1.5 ± 0.05	2.24 ± 0.075	24.99 ± 0.017
Juice	Early Stage	No Juice Formation	No Juice Formation	No Seed Formation
	Mid Stage	0.37 ± 0.075	3.75 ± 0.011	32.25 ± 0.05
	Late Stage	9.37 ± 0.075	0.87 ± 0.025	20.99 ± 0.025

Note: values are mean ± standard deviation. Means with superscripts having the same letters are not significantly different.

Table 2: DPPH Discoloration % and IC 50 values of methanolic extracts obtained from fruit residues of Kinnow mandarin.

Fruit Residue	Fruit ripening stage	DPPH Discoloration (%) ± SD	IC 50 mg\ml ± SD
Peel	Early Stage	86.4 ± 0.1	23.14 ± 0.025
	Mid Stage	80.2 ± 0.1	24.93 ± 0.03
	Late Stage	78.8 ± 0.1	25.41 ± 0.03
Pulp	Early Stage	86.13 ± 0.057	23.21 ± 0.011
	Mid Stage	78.53 ± 0.057	25.46 ± 0.017
	Late Stage	78.2 ± 0.1	25.57 ± 0.03
Seed	Early Stage	No Seed Formation	
	Mid Stage	71.5 ± 0.2	27.97 ± 0.08
	Late Stage	68.46 ± 0.057	29.2 ± 0.023
Juice	Early Stage	No Juice Formation	
	Mid Stage	83.9 ± 0.1	23.83 ± 0.03
	Late Stage	59.5 ± 0.1	33.61 ± 0.06

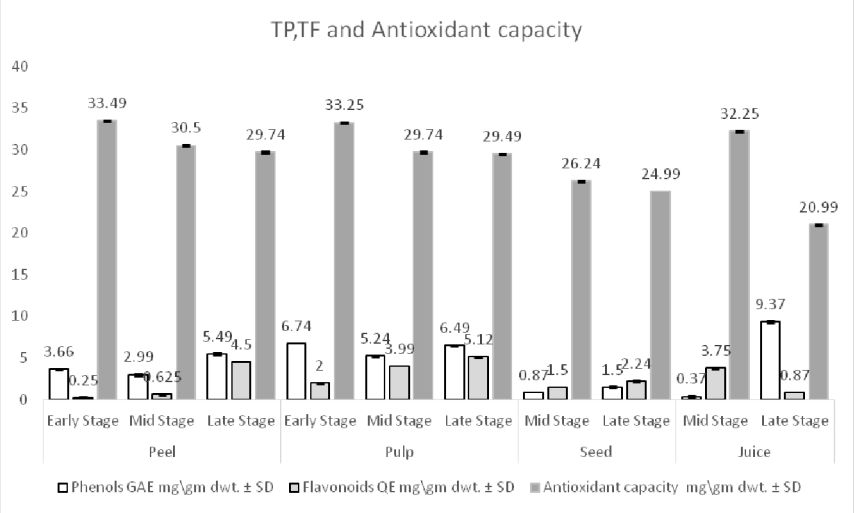

Fig. 1: phenolic content and antioxidant capacity of methanolic extracts obtained from fruit parts of Kinnow mandarin.

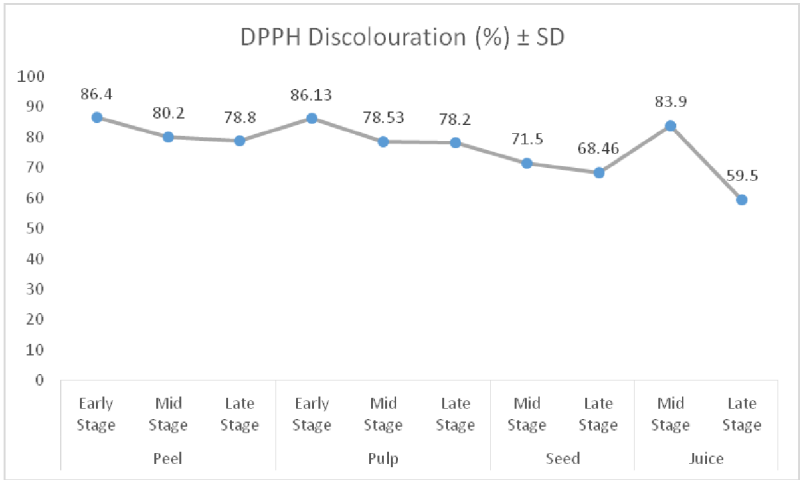

Fig. 2: DPPH Discoloration % of methanolic extracts obtained from fruit residues of Kinnow mandarin.

extraction methods of the individual laboratory. The values for average phenol content for immature and semi mature fruit in high to low order were found to be for early stage -pulp 6.74 > peel 3.66 and for mid stage - pulp 5.24 > peel 2.99 > seed 0.87 > juice 0.37.

Total flavonoid

The different parts of Kinnow fruit contains total flavonoid in varying amounts (Table 1).The TF varied from 0.25 mg QE/g dw (early stage peel) to 5.12 mg QE/g dw (late stage pulp). In all the ripening stages studied pulp has the highest flavonoid content. The average of values of concentration of Flavonoid in early stage fruit residues from high to low order was as follows : pulp early stage 2 >Peel early stage 0.25. The average of values of mid stage fruit residues flavonoids in decreasing order were: Pulp mid stage 3.99 > juice mid stage 3.75 > seed mid stage 1.5 > Peel mid stage 0.625. Flavonoid showed following pattern for late stage fruit residues in high to low order: Pulp late stage 5.12 > peel late stage 4.5 > seed late stage 2.24 > Juice late stage 0.87. The trend of TF results in ripened fruit is much lower than those obtained by Zhang et al (2018)), but was higher than those (3.14) published by Singh et al (2016) for late stage pulp. The variations in results might be due to the age of the plant, the rootstocks used for cultivation of Kinnow, environmental stress conditions and different sample matrices.

Antioxidant capacity analysis

As shown in Table 1, the antioxidant abilities for investigated extracts varied between 20.99 to 33.49 mg AA/g dw. The late stage peel and pulp residues have highest (29.74 and 29.49 mg AA/g dw respectively) whereas juice (20.99 mg AA/g dw) has lowest DPPH values. The average values of antioxidant capacity in ripened fruit from higher to lower order is as follows: peel (29.74 mg AA/g dw) > pulp (29.49 mg AA/g dw) > seed (24.99 mg AA/g dw)> juice (20.99 mg AA/g dw). In the early stage fruit the antioxidant capacity values in decreasing order is peel 33.49 > pulp

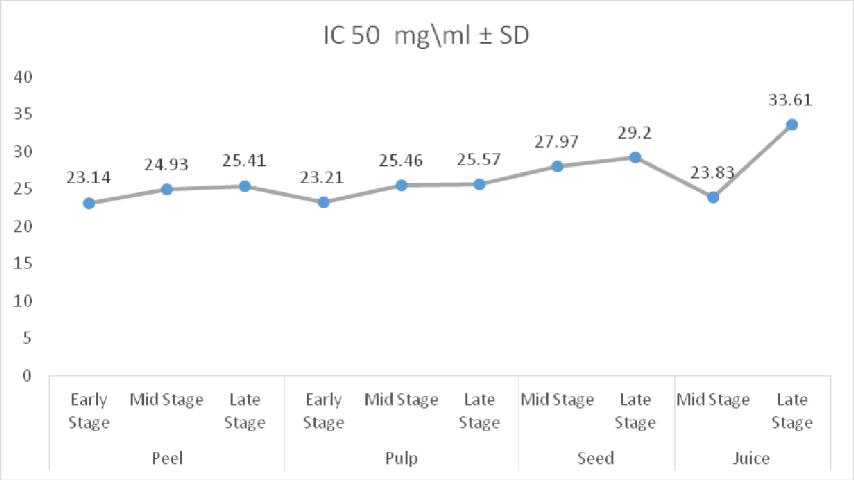

Fig. 3: IC 50 values of methanolic extracts obtained from fruit residues of Kinnow mandarin.

33.25 while in the mid stage fruit Juice contains the highest activity 32.25, followed by peel 30.5 and pulp 29.74. Mid stage seed showed the least antioxidant activity 26.24 for the stage.

Observations of present study indicate that compounds other than TP and TF could be involved in the antioxidant capacity of Kinnow mandarin. A large no. of studies have shown the presence of various antioxidants in Kinnow, which include Vitamins (A, C and E), Mineral elements (Se, Zn, Cu, Fe, and Mn), Phenolic compounds (Flavonoids, Phenolic acids, Coumarins), Terpenoids (Limonoids, Carotenoids) and Pectin (Zhou 2012, Ye 2005, Aggarwal and Michael 2014, Juhaimi *et al.*, 2016, Sidhu *et al.*, 2016, Aggarwal and Sandhu 2003, Sharma *et al.*, 2013). A number of these antioxidants (non-phenolic antioxidants) present in Kinnow might have been responsible for its antioxidant activity. There might be presence of interactions among various antioxidants (possible synergistic, additive and antagonistic interactions that may be observed when different natural antioxidants co-exist) as reported by Tsao (2015). At the same time, Phan *et al.*, 2016 reported that the combinations of two or more phytochemicals bring about changes in the ultimate biological effects. A number of mixtures of pure bioactive compounds or phytochemical-containing plant extracts provide synergy with regard to antioxidant status, anti-inflammation, anti-cancer and chemoprevention of several oxidative stress and metabolic disorders *in vitro*. Zou *et al.*, 2015 reported that in the DPPH method the results are influenced by many factors, such as antioxidants and interactions etc. All this may be the case with phenolic antioxidants of Kinnow and further research needs to be done in this regard to deal with the complexity of the issue.

Conclusion

Significant variation in TP, TF and antioxidant capacity between different extracts of Kinnow fruit residues indicated that efficacy of antioxidant with phenols vary considerably with fruit parts and ripening stages. With the ripening of fruit Phenols increase in seed and juice while fluctuate in peel and pulp, flavonoid increases in peel, pulp and seed; decreases in juice while in all the cases antioxidant activity decreases with time. Present research provided novel insight about variation of phenolic content and its antioxidant potential which may prove useful for future utilization of Kinnow fruit wastes. Further research is required for phytochemical and pharmacological investigation in this regard to separate the active compounds responsible of these biological activities and to understand the molecular mechanisms of action.

Abbreviations

IM – Immature, SM – Semi mature, CM – Commercially mature, GAE -Gallic Acid Equivalent, dw -dry weight, AA- Ascorbic Acid, DPPH-1,1 diphenyl-2-picryl hydrazyl

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