

Research Article

Effect of Mature Stages on Phenolic Contents, Antioxidant Activities and Mineral Distribution in Different Parts of Kei Apple Fruit

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Abstract: This study aimed to evaluate the concentrations of the heavy metals, macro and micro-elements as well as the distribution of phenolic constituents in different parts of half-ripe and full-ripe kei apple fruits. For mineral analysis, fruit samples were digested and analyzed using inductively coupled plasma optical emission spectrometry (ICPOES). The colorimetric determination of total polyphenolic contents (TPs) and the antioxidant capacity using DPPH and total antioxidant capacity (TAC) assays were done using aqueous alcoholic extracts. Also, the phenolic profiles were identified and quantified using high performance liquid chromatography (HPLC). The results showed that the highest antioxidant activity using DPPH, TAC, and total phenolic content were detected in the pulp of full-mature fruits and its values were 2.03 mg GAE/g d.w, 22.61 mg AAE/g d.w and 26.24 mg GAE/g d.w, respectively. The average of mineral concentrations in dried samples (in $\mu\text{g/g}$), ranged between: 6,164.99 to 18,339.61 (K); 2,538.06 to 3,488.92 (Ca); 749.26 to 2,110.12 (Mg); 82.13 to 733.36 (Fe); 22.74 to 58.02 (Zn); 6.43 to 11.85 (Mn); 10.17 to 20.02 (Cu); 1.86 to 8.73 (Ni) and much lower concentrations of Co, Pb and Cd. The key phenolic constituents of the fruit harvested in different times were 4-hydroxy benzoic acid, chlorogenic acid and catechol. Overall, harvesting of kei apple fruit in the first week of June indicates the full maturity of the fruit with characteristic higher phenolics, antioxidant capacity without any deterioration in most of the elemental minerals.

Keywords: kei apple fruit parts, harvest time, minerals, phenolic compounds, antioxidant capacity

1. Introduction

Kei-apple (*Dovyalis caffra*) is a tree belonging to the family Flacourtiaceae having a palatable fruit resembling apricot. The tree is native to the Kei River area, Eastern Cape, South Africa [1], while it is also abundant in other countries such as Egypt, Jamaica, Algeria, France, Italy, England, Australia and the United States of America. The cultivation of Kei-apple tree has probable profits as a critical element of rural-urban landscapes and infrastructure if it can be fruitfully incorporated into the physical and social fabric of towns and cities. Omotayo et al. [1] presented three broad classes of uses and abilities of the Kei-apple tree.

This unexplored fruit has a bitter acidic taste due to the presence of high amount of malic acid which may restrict its use as a fresh fruit and therefore usually directed to produce jams and preserves. In recent years, the fruit of Kei-apple was emerged in industrial products as alcoholic beverages [2]. The juice of the fruit has high ascorbic acid, phenolic constituents with a low pH level [3]. The characteristic yellow colour of the fruits might be explained by their richness

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of total carotenoids and specially β -carotene [4-5].

Flavonoids like luteolin, hesperidin, rutin, quercitrin, catechin and epicatechin have been identified in kei apple whole fruit [4]. Phenolic acids as p-coumaric, p-hydroxyphenyl acetic, 4-hydroxybenzoic, m-hydroxybenzoic, caffeic, 3-methoxy-4-hydroxyphenyl acetic, vanillic, protocatechuic, chlorogenic, rosmarinic, gallic, vanillic, ferulic, cinnamic and ellagic acids have been previously identified and quantified in kei apple whole fruit with different preparations [2-5]. Simple phenols like catechol and pyrogallol are found in the whole fruit of kei apple [4]. Also, the fruit extracts of kei apple were described to possess strong antioxidant capacity [4-5].

Additionally, the high nutritive value of Kei-apple fruit as a result for existence of considerable amounts of fructose (12-35.28 mg/g FW), glucose (1.21-15.06 mg/g), pectic constituents (3.7%) and proteins as well as the essential amino acids had been reported [5]. However, the potential toxicity of Kei apple entire fruit juice intraperitoneally injected in wean rats has been reported [6]. The recent pharmacological studies established that oral administration of Kei apple entire fruit juice failed to cause any deaths or alter of behavior in adult rats [4]. Additionally, the fruit extract did not cause any effect on kidney or liver injury and diminished the total cholesterol level and supplied protection against the cardiovascular diseases [4]. It could be suggested that orally administrated Kei-apple fruit aqueous extract at a dose up to 1000 mg/kg b.w/day failed to cause any harmful effects in adult rats. The antimicrobial, antiviral and anticancer activities of Kei apple entire fruit extract have been recently reported [7]. Also, it has been reported by [8-9] that Kei-apple fruit extracts has the ability to synthesize bioactive metal nanoparticles.

To the best of our knowledge, information about nutritional values of kei apple fruit from Egypt at different ripening stages is scarce. So, the present work aims to characterize the phenolic profile, antioxidant capacity and the mineral composition of skins, pulp and seeds cultivated in Egypt to determine the adequate date of maturity from a nutritive view.

2. Materials and methods

2.1 Chemicals

Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH \bullet), gallic, coumaric, 4-OH benzoic, syringic, vanillic, caffeic, protocatechuic and ferulic acids as well as rutin, quercetin, luteolin, hydroxytyrosol, oleuropein, catechol, tyrosol, pyrogallol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Nitric acid, ascorbic acid (AA), methanol and hydrogen peroxide (35%, analytical grade) were obtained from El-Nasr Company for pharmaceuticals.

2.2 Sampling

A weight of 500 g of half ripe kei apple fruits (80% yellow, with a diameter that ranged between 2.75 and 3 cm) harvested in the third week of May as well as 500 g of the fully ripe ones (100% yellow, with a diameter that ranged between 3 and 3.5 cm) harvested at the second week of June were obtained from a particular farm in Belbis, El-Sharkia Governorate, Egypt (31.11667 °N 30.63333 °E) during the period of May-June 2020. The fruits from each maturity stage were then carefully washed with running tap water for 3 min followed by drying at room temperature. The skins of the fruits were removed by hand-peeling and cut up into small pieces. Pulp containing seeds were manually dissociated and then cut up into smaller pieces. The obtained seeds were washed with distilled water. Plastic knives were used for cutting and homogenization of kei apple samples to avoid metal contamination. Then, each fruit part from each harvest time was lyophilized (Christ, Osterode, Germany). Lyophilized portions of kei apple fruits (skin, pulp and seeds) were ground individually (Retsch, Haan, Germany) and passed through a 100-mesh sieve before analysis. The resultant powders were stored at -20 °C till HPLC and ICP-OES analysis and to prepare the extracts.

2.3 Determination of mineral concentrations

Lyophilized samples (0.3 g) of kei apple were firstly digested with 5 mL 65% nitric acid plus 3 mL hydrogen peroxide (35%, analytical grade) in microwave digestion system Speedwave®, Berghof, Germany. Digested solutions were diluted with ultrapure water to a volume of 100 mL. In this study, the level of the elements (K, Ca, Mg, Fe, Zn, Cu, Co, Mn, Ni, Pb and Cd was recorded with the use of an Inductivity coupled plasma (Thermo Scientific™ 158 iCAP™

7,000 Plus Series ICP-OES). The levels of the examined elements in kei apple samples were estimated by the equivalent standard calibration curves done by using standard solutions of the elements of interest. Triplicate analyses were done on each tested sample.

2.4 Phytochemical properties

2.4.1 Preparation of kei apple bioactive supernatants

The extraction procedure of the bioactive components from kei apple samples was done according to Taher et al. [10]. In detail, 10 grams of each part of fresh kei apple fruit were macerated overnight with 50 ml of 80% methanol. After centrifugation at 5,000 × g for 15 min at 4 °C, supernatants were collected, and kept at -20 °C till analysis. The previous supernatants were used for the determination of TPs and antioxidant capacity. For HPLC analysis, the previous supernatants were evaporated using a rotary evaporator at 45 °C until the weight of the evaporated filtrate was less than 10% of the original weight of the filtrate. Then the residual water in concentrates was detached by lyophilization.

2.4.2 Colorimetric determination of total polyphenols

Total polyphenolic contents of kei apple extracts were estimated by using the Folin-Ciocalteu method as described by Taher et al. [10] Gallic acid (25-150 µg/ml) was chosen for the plotting of the standard curve for total polyphenols. It was expressed as µg gallic acid equivalent per g dry weight (µg GAE/g).

2.4.3 HPLC analysis

Determination of phenolic compounds was carried out using Agilent 1,260 infinity high-performance liquid chromatography (HPLC, Agilent, USA) equipped with quaternary pump and variable wavelength detector (VWD) set at 284 nm. Before analysis, the lyophilized supernatants were dissolved in methanol and filtered through a 0.45 µm PTFE filter, and then injected in a volume of 20 µL. The separation of phenolic constituents was done on a Kintex-R 5 µM EVO C18 (4.6 mm, × 100 mm, Phenomenex, USA), at a temperature of 30 °C. A binary solvent mixture involves water acidified with 0.2% H₃PO₄ (solvent A) and acetonitrile/ methanol 1:1 (solvent B) was used as gradient *elution system* in a constant flow rate (1 mL/min). A linear gradient was run from 96% (A) and 4% (B) to 50% (A) and 50% (B) during 40 min; during 5 min, it changed to 40% (A) and 60% (B); and finally 0% (A) and 100% (B), for 15 min [11]. All phenolic compounds were recognized by matching their retention times with those of reference phenolic ingredients (coumaric acid, p-OH benzoic, syringic acid, gallic acid, vanillic acid, caffeic acid, protocatechuic acid, ferulic acid, rutin, quercetin, luteolin, hydroxytyrosol, oleuropein, catechol, tyrosol, pyrogallol). Each compound was quantified according to the peak area measurements, which were reported in calibration curves of the corresponding standards.

2.4.4 Antioxidant activity

2.4.4.1 2,2-diphenyl-1-picrylhydrazyl (DPPH•) free radical scavenging activity assay

Radical scavenging potential of supernatant extracts by DPPH was measured in three replicates according to the method written by Taher et al. [10] with slight modifications. Each test sample consisted of 0.5 ml of Kei apple homogenate or gallic acid (2.5-50 µg/ml) solutions and 2.0 ml of freshly prepared methanolic DPPH solution (0.06 mM). The mixture was placed in dark for 30 min at room temperature. The absorbance of each sample was recorded at 517 nm using spectrophotometer. Control tube consisted of 0.5 ml of methanol and 2.0 ml of the radical solution. The percentage of inhibition of DPPH scavenging activity was calculated using a regression equation between the concentration of gallic acid and DPPH inhibition %, and the results were indicated as mg gallic acid per gram dry weight (mg GAE/g DW).

2.4.4.2 Total antioxidant capacity (TAC)

The total antioxidant capacity of kei apple supernatants was assessed by phosphomolybdate method as stated by Haddouchi et al. [12] in three replicates. In detail, 0.5 ml of each of kei apple supernatants or AA (25-200 µg/ml) was mixed with 1 ml of the coloring reagent solution containing 28 mM disodium hydrogen phosphate, 4 mM ammonium

molybdate and 0.6 M sulphuric acid. The mixture was kept in boiling water bath for 75 min. After cooling, absorbance was recorded at 765 nm against blank which was prepared by adding 0.5 ml of 80% methanol instead of the supernatant. Standard curve was prepared using different AA absorbance values. TAC of kei apple supernatants were expressed as mg ascorbic acid equivalent (AAE) per gram dry weight.

2.4.5 Statistical analysis

All data are presented as mean \pm standard error (SE). Tukey's HSD Test was performed using one-way analysis of variance (ANOVA) by SAS Software (version 9.1, SAS Institute, Cary, NC, USA). The correlation analysis results of TP with TAC, DPPH and K were expressed as Pearson correlation coefficients using SPSS Version 24.0 (SPSS, Chicago, IL, USA). The slope of the calibration curve and the coefficient of determination (R^2) were obtained by using MS Excel 2,010 from Microsoft (Redmond, WA, USA).

3. Results and discussion

3.1 Macro-elements

The concentrations of the macro elements in each *Kei apple* samples are presented in Figure 1. The result of each element displays the average \pm standard error of 3 replicates. Quantities of the macro elements varied extensively in different parts of *Kei apple* fruit. As expected, K, Ca and Mg had the highest concentrations in each part of *Kei apple* fruit. It is detected that among all the minerals estimated in the tested samples, potassium accumulation was the highest in kei apple than other elements. In general, the highest significant concentration of K (18,339.61 $\mu\text{g/g}$) was found in the pulp of *Kei apple* fruit harvested on time, while the lowest amount of K was found in the seeds of the fruit harvested 3 weeks earlier (6,164.99 $\mu\text{g/g}$; Figure 1A). The levels of K in different parts of the fruit were significantly altered during the last step of maturation (Figure 1A). Overall, the high obtained concentrations of potassium in *kei apple* different parts provided that this fruit may be considered a good source of potassium, which is vital to maintain acid balance and body water content [13]. The greater K intake was related with a slighter risk of stroke [14].

Calcium is a principal constituent of teeth and bones also is responsible for regulation function of muscle and nerve [15]. High concentration of available calcium for absorption could help patients with chronic renal failure to avoid abnormal release of calcium from the bone as a result of low serum calcium [16]. In this study, the highest significant concentration of Ca (3,488.92 $\mu\text{g/g}$) was found in the skin of half-ripe *Kei apple* fruit (Figure 1B). Similarly, the skin of prickly pear fruit accumulated higher concentration of Ca than those found in the pulp or the seeds [17]. Remarkably, during the last 3 weeks of ripening, the level of Ca was significantly decreased in the skins (3,488.92 vs 3,043.29 $\mu\text{g/g}$) as well as in seeds (3,203.19 vs. 2,538.06 $\mu\text{g/g}$).

As shown in Figure 1, the distribution of Mg among the fruit parts in this study follows the order: -seeds $>$ skin $>$ pulp. This order has been previously reported in the fruit of the Brazilian Cerrado Plant [18]. The seeds of *Kei apple* fruit harvested 3 weeks earlier contained the maximum concentration of Mg (2,110.12 $\mu\text{g/g}$), its concentration was significantly decreased to be (1,814.19 $\mu\text{g/g}$) during the last step of maturation. Likewise, a significant decrease of Mg was happened in fruit skin during the last step of maturation (Figure 1C). Remarkably, the flesh part of full mature kei apple fruit had a slightly higher content than that of half-ripe fruits. Overall, the significant alternations of Mg levels in all parts of *Kei apple* fruit during the last step of maturation agreed with the previous findings concerning different parts of *Musa paradisiaca* fruit in relation to the degree of maturation [19].

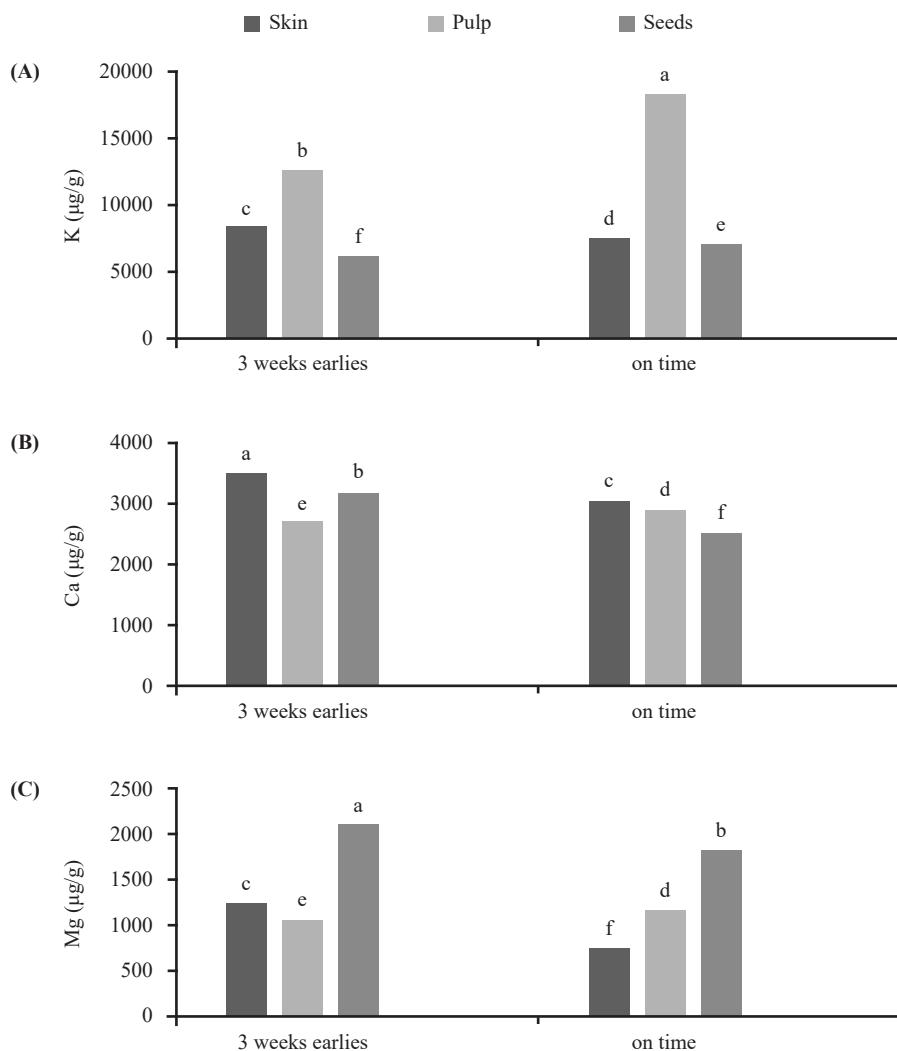


Figure 1. concentrations of potassium (K), calcium (Ca), magnesium (Mg) of different parts of Kei apple fruit harvested at two different times. Data are shown as mean \pm SE of three replicates. Significant difference between the results was calculated by using analysis of variance (ANOVA) with Duncan's multiple range test at significance level of $p < 0.05$

3.2 Micro-elements and heavy metals

As noticed from Figures (2) and (3), the order of richness of the vital micronutrients was as follow: -Fe > Zn > Cu > Mn > Ni > Co. Iron is a necessary nutrient for humans and its enough supply is very important especially for females (15-50 years old) and for babies in the first year of life [20]. As shown in Figure (2A), variable concentrations of Fe were found in kei apple samples which ranged between 82.13 to 733.36 $\mu\text{g/g}$. The skin of the fruit had the highest significant contents of Fe regardless the harvest day; its value was 733.36 $\mu\text{g/g}$ (Figure 2A) for that harvested 3 weeks earlier. However, a sharp decline in Fe level of ripe fruit skins (360.85 $\mu\text{g/g}$) was observed. The concentration of Fe was increased with no significant in the pulp of Kei apple during the last step of maturation (Figure 2A). The concentrations of Fe in the seeds of half-ripe and ripe fruits were 82.13 and 87.58 $\mu\text{g/g}$, respectively. Overall, this study revealed that the skins of kei apple fruit represent a rich source of iron especially in half-ripe fruits.

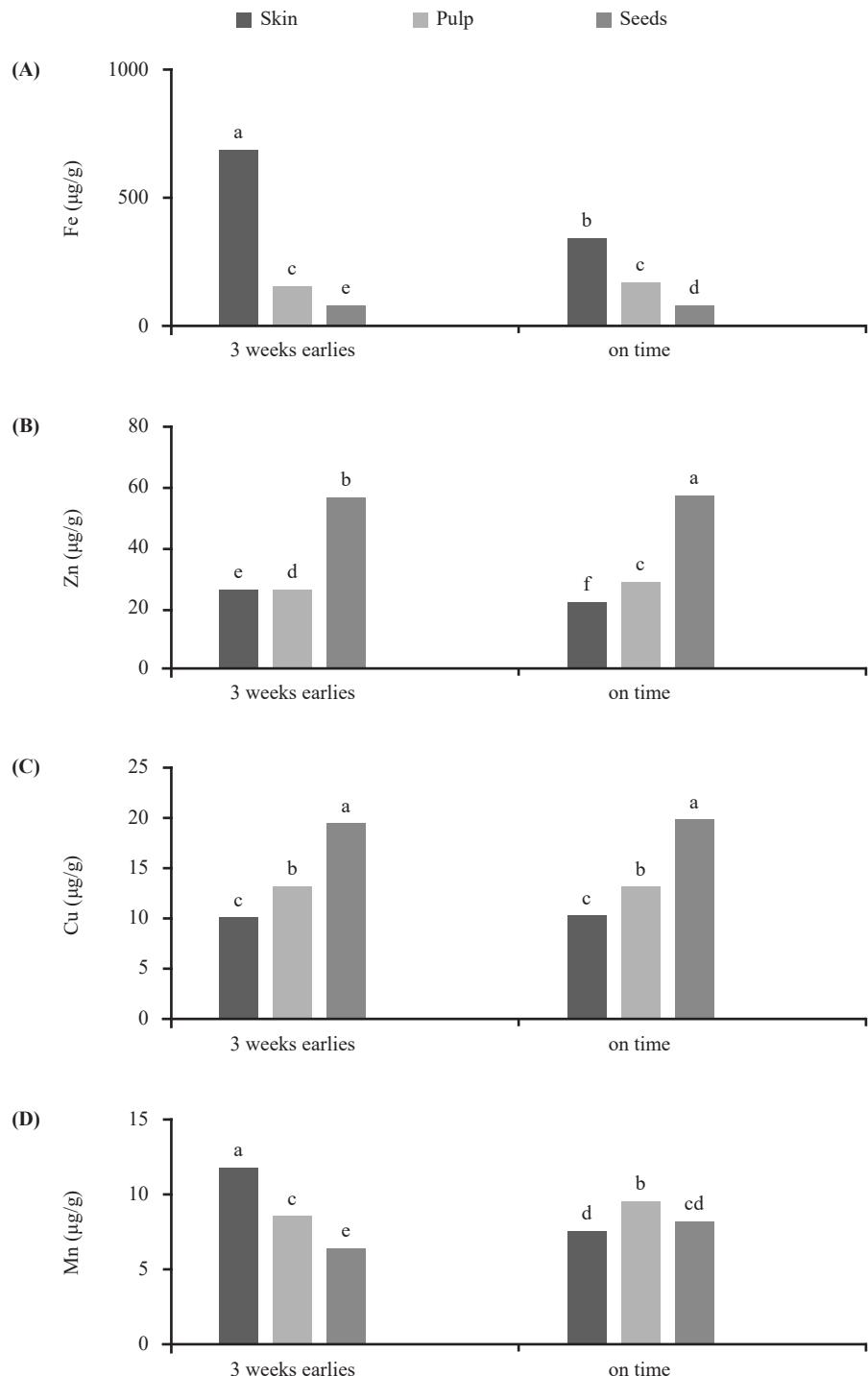


Figure 2. concentrations of iron (Fe), zinc (Zn), copper (Cu) and manganese (Mn) of different parts of Kei apple fruit harvested at two different times. Data are shown as mean \pm SE of three replicates. Significant difference between the results was calculated by using analysis of variance (ANOVA) with Duncan's multiple range test at significance level of $p < 0.05$

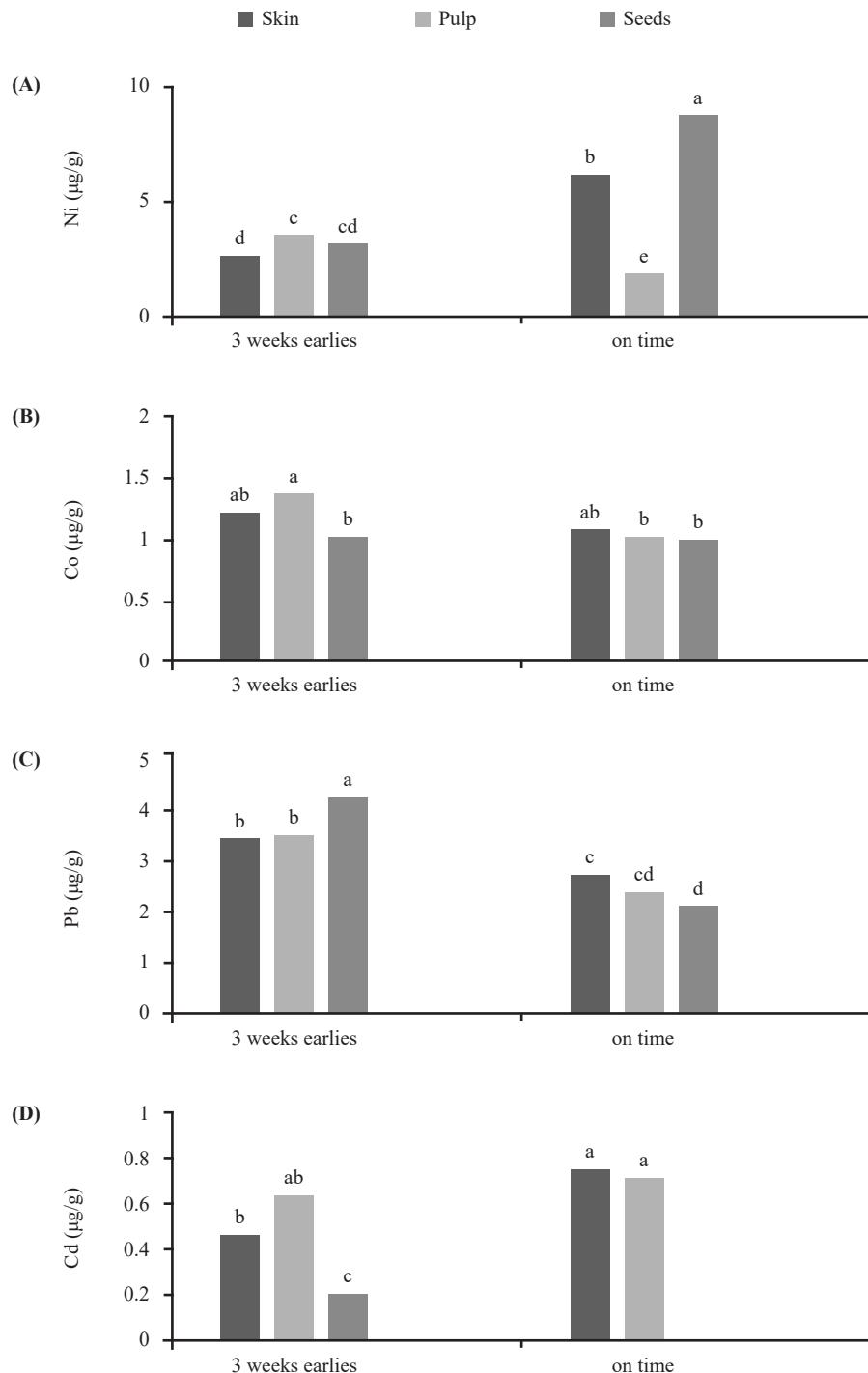


Figure 3. concentrations of nickel(Ni) cobalt (Co), lead (Pb) and cadmium (Cd) of different parts of Kei apple fruit harvested at two different times. Data are shown as mean \pm SE of three replicates. Significant difference between the results was calculated by using analysis of variance (ANOVA) with Duncan's multiple range test at significance level of $p < 0.05$

Zinc is a vital element in the metabolism of prostaglandins, neurotransmitters, and for preserving brain structure and function. In fact, recent researches showed the proper impact of zinc in the treatment of hyperactivity complaints in children [21]. Moreover; zinc is a suitable remedy in the dealing of some disorders, such as cancer and pro-inflammatory conditions [22]. In Figure (2B), the amount of Zn noticed in kei apple fruit samples ranged between 22.74 to 58.02 $\mu\text{g}/$

g. The maximum concentration of Zn was found in the seeds of *Kei apple* fruit, where its values were 56.80 and 58.02 $\mu\text{g/g}$ in half-ripe and ripe fruits, respectively. This finding agreed in a large extent with that observed by El-Kossori et al. [17] who noted that the seeds of prickly pear fruit contained reasonably higher amount of Zn than those found in skin and flesh pulp. Also, the level of Zn increased significantly in Kei apple fruit pulp and seeds during the last step of maturation (Figure 2B), similar observations have been reported in tomato fruit [23]. Overall, one of the outcomes of this study validated that kei apple fruit is considered as an excellent source of zinc for children and adults.

Copper is a coenzyme required for redoxchemical cytochrome oxidase and consider as a critical cofactor in collagen amalgamation, iron utilization and concealment of free radicals [16]. As shown in Figure (2C), the amount of Cu detected in kei apple fruits ranged between 10.17 to 20.02 $\mu\text{g/g}$. The maximum concentration of Cu was found in the seeds of *Kei apple* fruit, its values were 19.52 and 20.02 $\mu\text{g/g}$ in half-ripe and ripe fruits, respectively (Figure 2C). Similarly, the accumulation range (6.57-9.00 $\mu\text{g/g D.W}$) of Cu in the seeds of some grape cultivars harvested in 3 different times was higher than that found in the pulp (0.37-1.00 $\mu\text{g/g D.W}$) [24]. Additionally, the order of Cu accordance in kei apple was as follow: -seeds > pulp > skins. Figure (2C) displayed a non-significant alteration in the level of Cu during the last step of maturation in all parts of kei apple fruit, results are differed to the work by Özcan et al. [24].

Manganese is a cofactor of some enzymes essential for glycoprotein synthesis and critically affects the activity of superoxide dismutase [16]. Manganese is an important element for nutrition need and possibly toxic for human health in high levels [25]. In this study, the maximum concentration of Mn was noted in the skins of *Kei apple* fruit harvested 3 weeks earlier (11. 85 $\mu\text{g/g}$), similar finding has been reported in prickly pear fruit [17]. However, its concentration was significantly decline in ripe fruit to be 7.57 $\mu\text{g/g}$. Contrarily, the concentrations of Mn were significantly increased in the fruit pulp as well as the seeds during the last step of maturation (Figure 2D). Overall, this study exhibited significant alterations in the concentrations of Mn during the last step of maturation in all parts of kei apple fruit, findings are related to the work by Rogiers et al. [26].

In Figure (3A), the amount of nickel seen in kei apple fruit samples ranged between 1.86 to 8.73 $\mu\text{g/g}$. The unique role for Co is an ingredient of vitamin B₁₂ [27]. So, it is an important element for homeostasis of heme, DNA, fatty acids and amino acids [27]. As shown in Figure (3B), the amount of Co detected in kei apple fruit parts ranged between 0.83 to 1.38 $\mu\text{g/g}$. Also, this study exhibited that the levels of Co were decreased in all parts of kei apple during the last step of maturation with some significance. These findings agreed in a large extent with those detected by Bressy et al. [23] who noted that organic cultivation of early matured tomato fruits had higher significant content of Co that of full-matured ones.

3.3 Heavy metals

Contrarily to macro and essential trace elements, the heavy metals lead and cadmium keep no nutritional value. Figure 3 shows also the level of the toxic heavy metals in kei apple fruit samples which indicated the presence of low amounts of lead (2.10-4.25 $\mu\text{g/g}$, Figure 3C) and cadmium (0.00-0.75 $\mu\text{g/g}$, Figure 3D). Several reports have documented the probable contamination of the dried fruits with Pb and Cd [28-29]. The observed concentrations of Pb and Cd in the tested samples of kei apple fruit in the present study may be ascribed to the existence of heavy metal in close soil geochemistry.

3.4 Total polyphenols (TPs)

Previous studies have exposed that kei apple whole fruit had high polyphenolic content particularly phenolic acids [2-5]. Figure 4A displays the evolution of TPs content in kei apple fruit different parts i.e. skin, pulp and seeds during the last period of maturation. TPs content of Kei apple fruit samples ranged between 11.67 to 26.24 mg GAE/g d.w. (Figure 4A), lower range of TP had been reported [30]. The fruit pulp of ripe fruit contained the highest amount of TPs as 26.24 mg GAE/g d.w., while skins of half-ripe fruit had the lowest concentration of TPs as 11.67 mg GAE/g d.w. Overall the levels of TP in different parts of the fruit were significantly increased during the last step of maturation (Figure 4A). In other words, our results proved that the dynamics of TPs for different parts of kei apple cultivated in Egypt were directly correlated with ripening stage and presented a significant increase in TPs during maturation. To the best of our knowledge, no previous researches estimated TPs content during maturity stages.

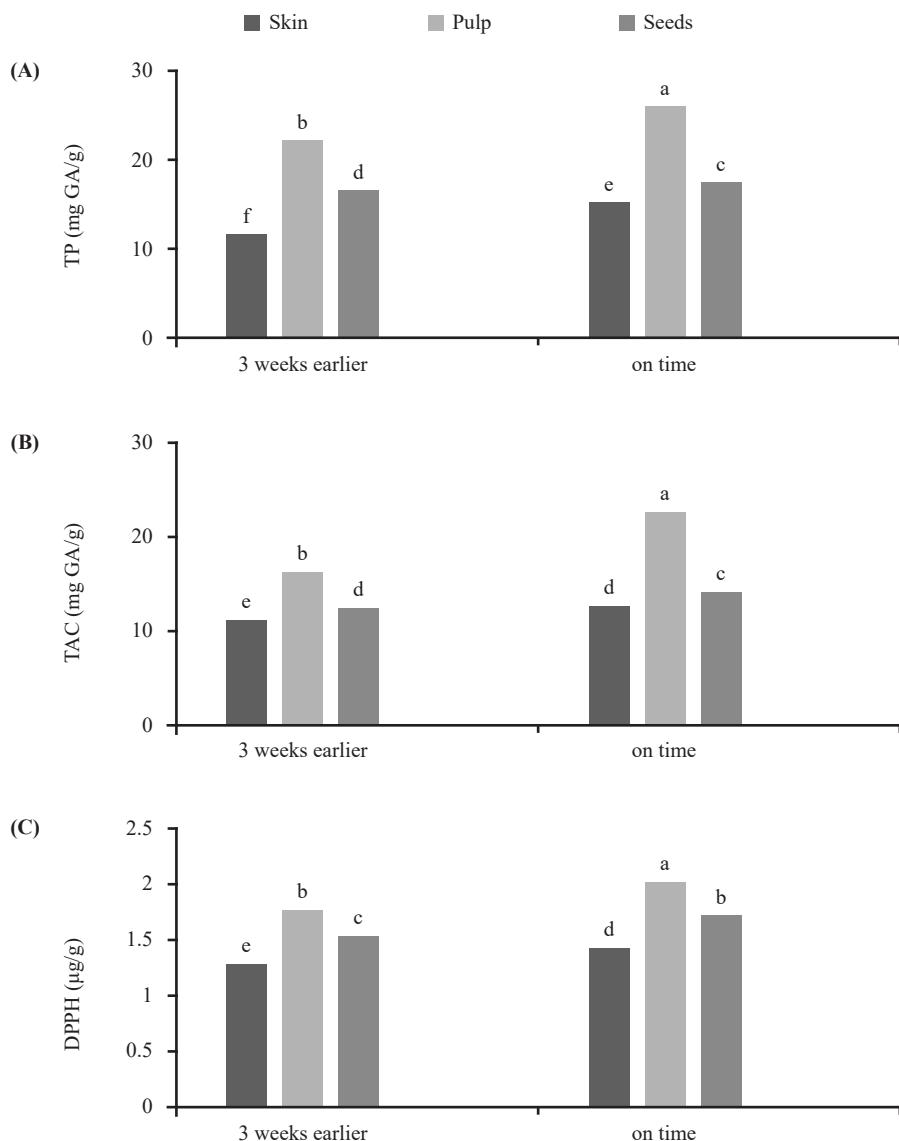


Figure 4. Total polyphenols (TP), Total antioxidant capacity (TAC) and DPPH scavenging ability of different parts of Kei apple fruit harvested at two different times. Data are shown as mean \pm SE of three replicates. Significant difference between the results was calculated by using analysis of variance (ANOVA) with Duncan's multiple range test at significance level of $p < 0.05$

3.5 Phenolic profile

Figures (5-7) and Table (1) shows the chromatograms of HPLC analysis and the concentration of different phenolic constituents of kei apple fruit different parts harvested at two different harvest times. Chlorogenic acid was the predominant phenol in the skin of kei apple, where its values were 5,737.18 and 7,470.07 $\mu\text{g/g}$ in half mature and full mature fruits, respectively (Table 1). Additionally, 4-hydroxybenzoic acid (4HBA) was the second major phenol in the skin of kei apple where its values were 520.86 and 2,212.23 $\mu\text{g/g}$ in half mature and full mature fruits, respectively. Overall, chlorogenic acid, ferulic acid and myricetin were only accumulated in the skin of the fruit. Similarly, chlorogenic acid was identified in whole fruit extract of kei apple in high amount [4]. It could be suggested that the richness of kei apple fruit with chlorogenic acid was due to the richness of its skin with a significant amount of this particular phenolic acid. To the best of our knowledge, no previous researches evaluated the phenolic profiles of kei apple fruit different parts.

Table 1. The concentration of phenolic compounds of Kei apple fruit different parts harvested at two different times by HPLC.

Phenolic compound ($\mu\text{g/g DW}$)	Skin		Flesh pulp		Seeds	
	Half-ripe	Full-ripe	Half-ripe	Full-ripe	Half-ripe	Full-ripe
Phenolic acids						
Gallic acid	235.77	155.55	0.00	283.93	0.00	0.00
4-hydroxybenzoic	520.86	2,212.93	6,267.20	8,356.56	2,963.38	4,969.18
Chlorogenic acid	5,737.18	7,470.07	0.00	0.00	2.05	0.00
Vanillic acid	0.00	0.00	42.59	56.50	57.48	58.85
Caffeic acid	0.00	6.98	3.25	3.89	9.84	3.25
Syringic acid	190.80	36.82	9.40	33.03	21.35	6.84
p-coumaric acid	0.00	0.00	0.00	1.96	3.24	3.30
Ferulic acid	91.55	124.13	13.16	0.00	0.00	8.72
Benzoic acid	0.00	0.00	108.45	231.64	0.00	178.30
Ellagic acid	0.00	0.00	0.00	0.00	0.00	
Rosmarinic acid	0.00	0.00	61.91	0.00	0.00	51.93
Simple phenols						
Catechol	108.00	215.73	1,425.19	1,846.14	92.91	809.72
Pyrogallol	8.87	5.04	3.43	17.34	670.32	2.13
Flavonoids						
Rutin	91.61	0.00	0.00	0.00	0.00	0.00
Myricetin	121.11	66.98	0.00	0.00	0.00	0.00
Quercitin	0.00	0.00	38.98	0.00	36.95	34.32
Kampherol	12.19	14.17	10.28	0.00	0.00	10.47
Naringein	0.00	161.94	0.00	0.00	0.00	0.00

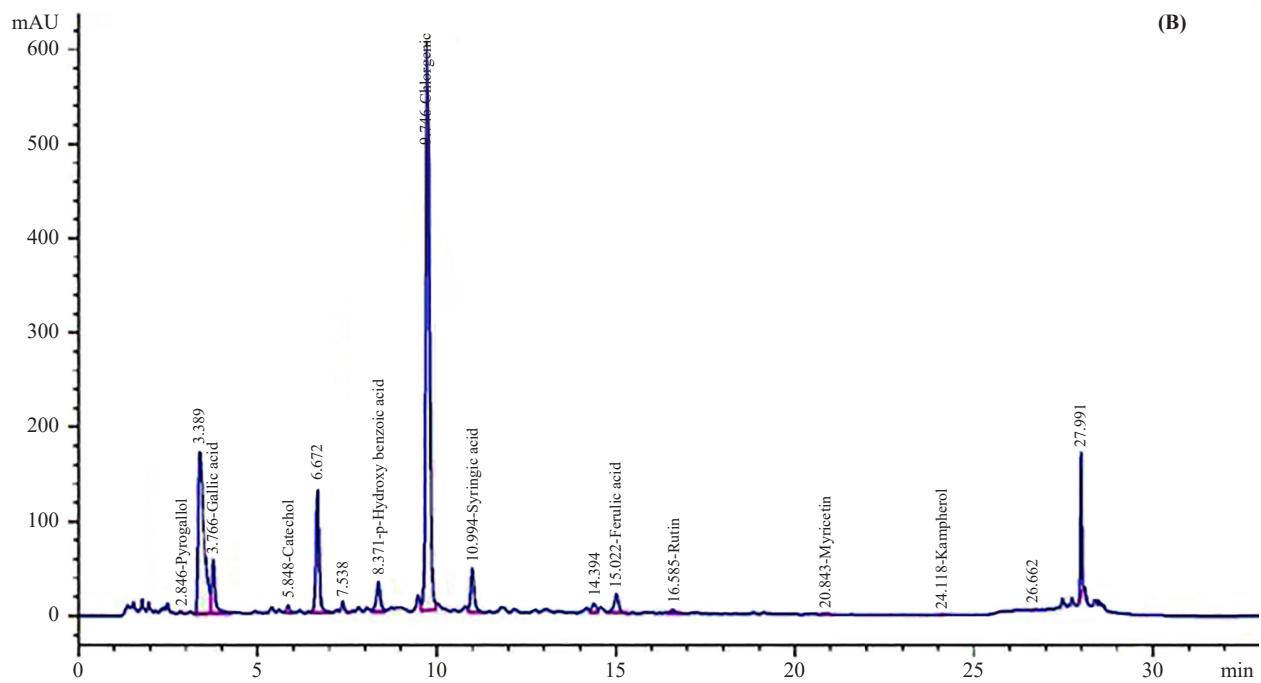
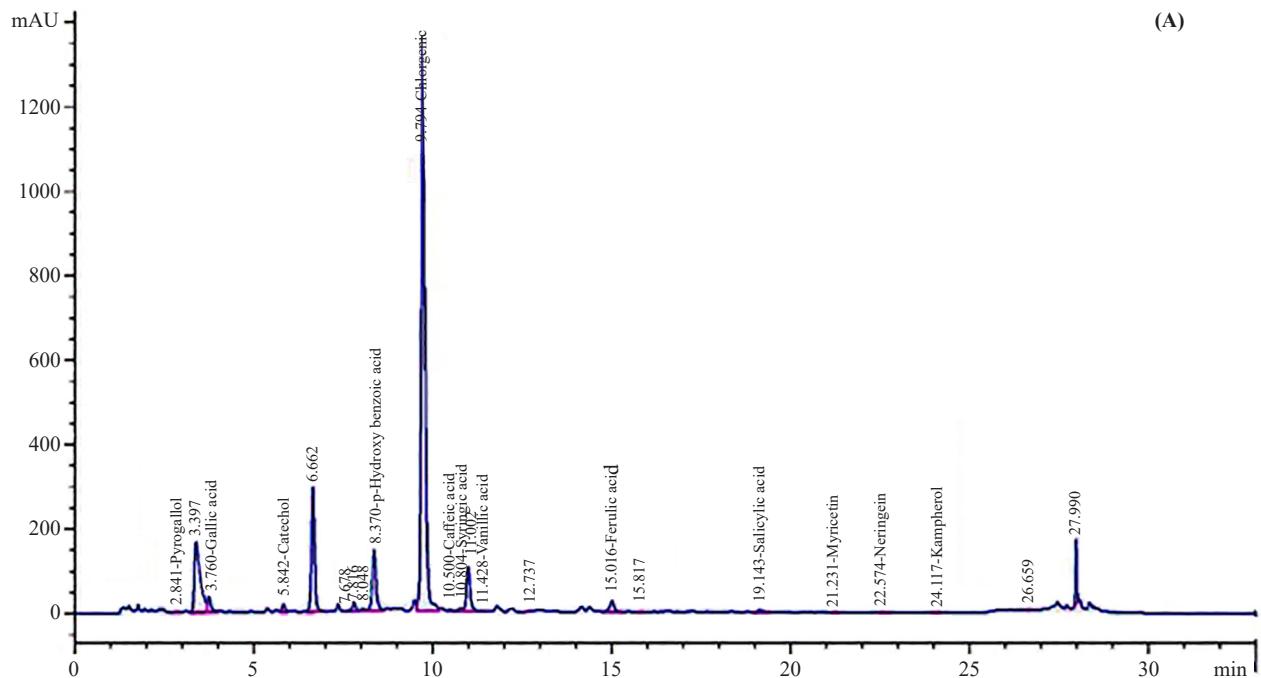


Figure 5. HPLC chromatograms of skins of kei apple fruits harvested 3 weeks earlier (A) and harvested on time (B).

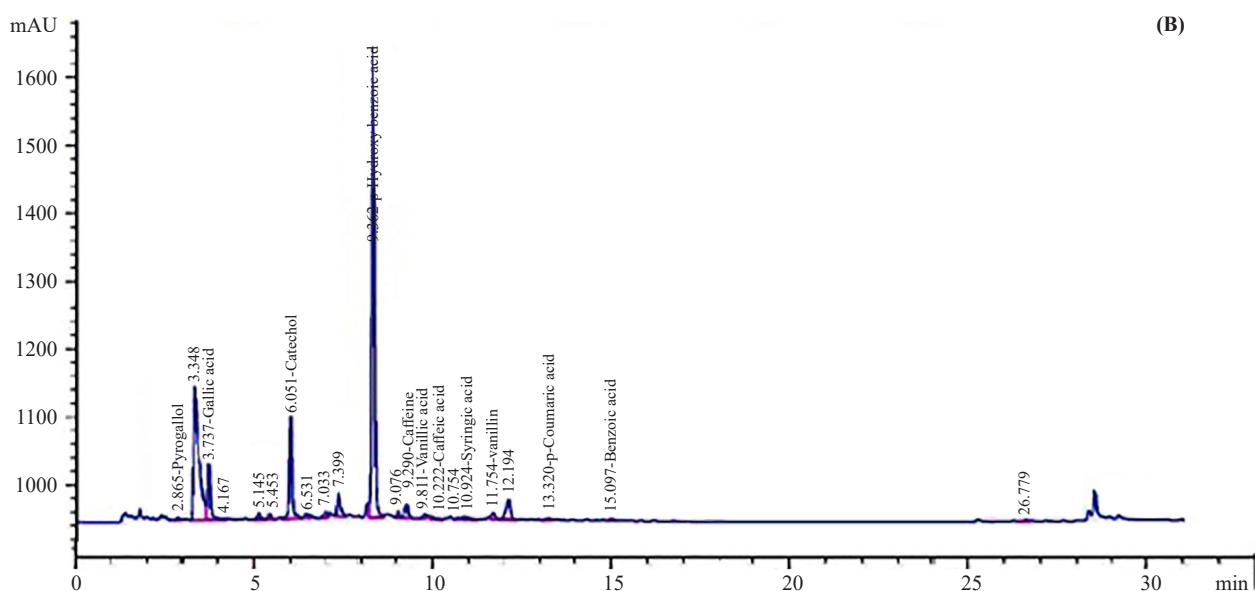
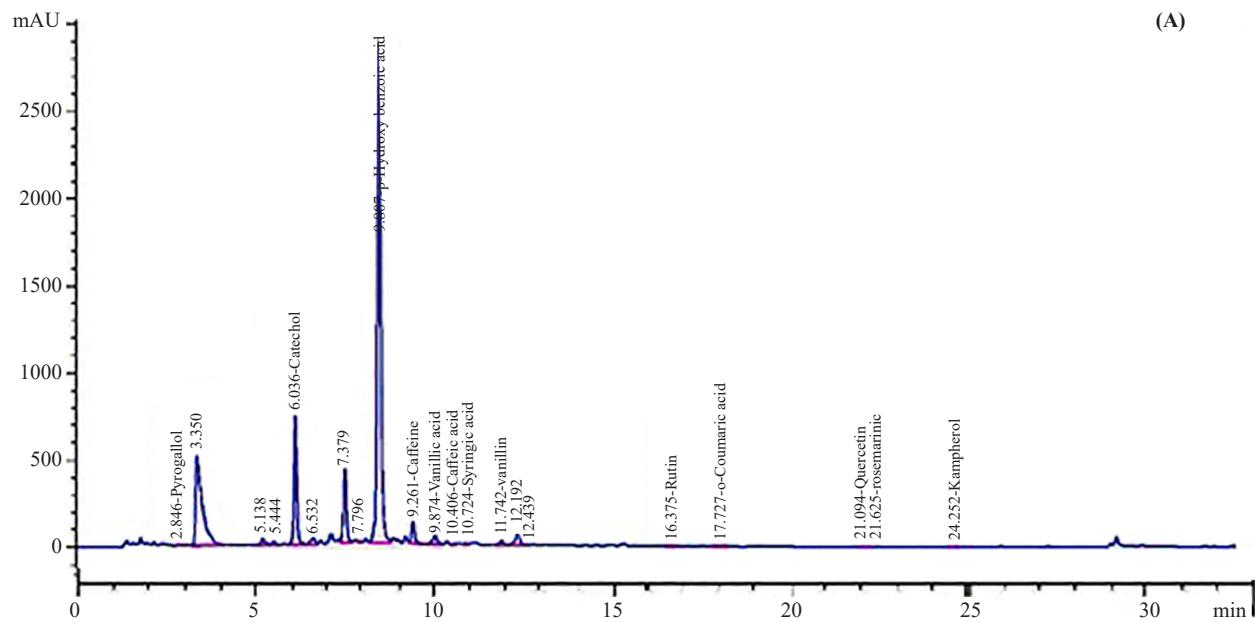


Figure 6. HPLC chromatograms of flesh pulp of kei apple fruits harvested 3 weeks earlier(A) and harvested on time (B).

4-Hydroxy benzoic acid (4HBA) is a bioactive phenolic acid which usually persists in many plants including coconut, carrots, grapes and numerous of plant leaves [31-33]. While, the literature data of kei apple fruit revealed the presence of a trace amount of meta-hydroxybenzoic acid [30]. The order of richness of 4HBA regardless harvest day was as follow: -flesh pulp > seeds > skins. In other words, HPLC chromatograms in the present study showed that 4-hydroxybenzoic acid (4HBA) was the predominant phenolic constituent in the flesh pulp and the seeds of Kei apple fruit (Figures 6 and 7). Table (1) shows the distribution of the aforesaid phenol in different parts of half-ripe and ripe fruits of kei apple. 4HBA was the predominant phenol in flesh pulp of half mature and mature fruits in values of 6,267.20 and 8,356.56 μ g/g, respectively. While, the seeds of the fruit had lower concentrations of 4HBA as 2,963.38 and 4,969.18

µg/g in half mature and mature fruits, respectively. Remarkably, the levels of 4HBA in different parts of the fruit were notably increased during the last step of maturation. Overall, biotic or abiotic stress leads to the activation of an enzyme or group of enzymes downstream from PAL that convert cinnamic acid to 4HBA like cinnamic acid 4-hydroxylase [34-35]. Additionally, treatment of plant cells with an elicitor can cause a transient increase in PAL activity, followed by an increase in 4HBA covalently bound to cell walls [36].

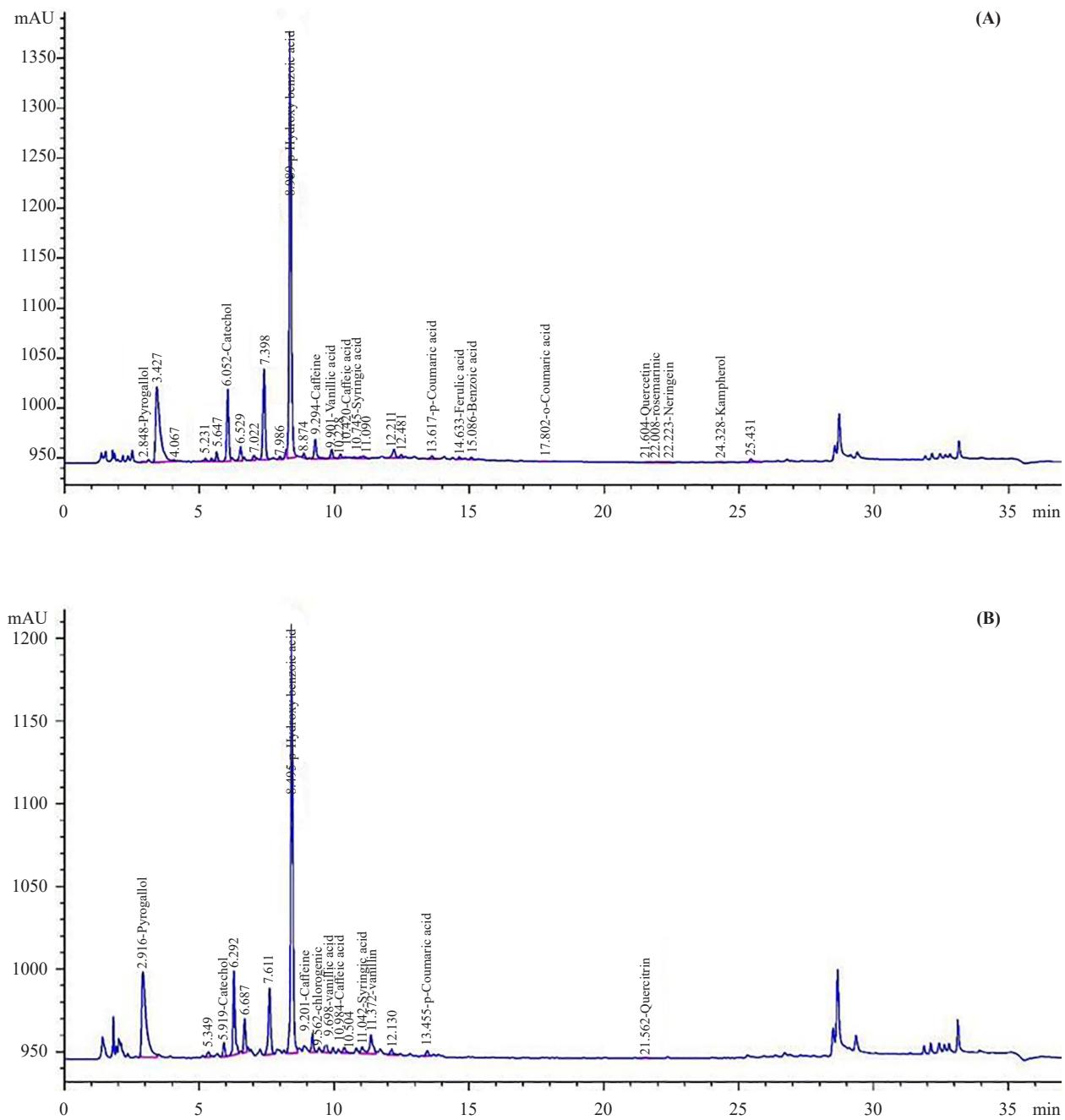


Figure 7. HPLC chromatograms of the seeds of kei apple fruits harvested 3 weeks earlier (A) and harvested on time (B).

Table (1) shows the levels of catechol as the second principal phenol in the fruit pulp and the seeds of kei apple

fruit when harvested at different times. Remarkably, the levels of catechol in kei apple fruit different parts were notably increased during the last step of maturation. The maximum concentration of catechol was found in the pulp of Kei apple fruit, where its values were 1,425.19 and 1,846.14 $\mu\text{g/g}$ in half-ripe and full-ripe fruits, respectively. The order of richness of catechol regardless harvest day was as follow: -flesh pulp > seeds > skins. Catechol and other benzenols like pyrogallol are commonly existed in many fruits, vegetables, grains, areca nut, coffee, tea, beer, and wine [37]. The present study exposed that catechol was the second principal phenolic constituent in the pulp and the seeds of Kei apple fruit. However, Taher et al. [4] found that pyrogallol was the second major phenol in kei apple whole fruit simultaneously with a diminished level of catechol. Overall, catechol and pyrogallol belonging to phenolic compounds; are allelochemicals synthesized by the shikimate pathway in plants [38].

HPLC analysis revealed also that kei apple samples in this study contained much lower amounts of vanillic acid (0-58.85 $\mu\text{g/g}$), pyrogallol (2.13-670.32 $\mu\text{g/g}$), gallic acid (0-283.93 $\mu\text{g/g}$), syringic acid (6.84-190.80 $\mu\text{g/g}$), ferulic acid (0-124.13 $\mu\text{g/g}$), myricetin (0-121.11 $\mu\text{g/g}$), rosmarinic acid (0-61.91 $\mu\text{g/g}$), quercetin (0-38.98 $\mu\text{g/g}$) and caffeic acid (0-9.84 $\mu\text{g/g}$).

3.6 Antioxidant activity

Figure (4) shows the antioxidant activity of kei apple samples estimated using TAC and DPPH assays. In TAC assay, its values for the fruit samples ranged between 11.06 and 22.61 mg AAE/g d.w (Figure 4B). Keeping in mind, TAC of kei apple for different parts increases significantly with the maturity advance. Moreover, TAC of the fruit parts followed the order flesh pulp > seeds > skins. The flesh pulp of fruit harvested on time recorded the highest TAC as 22.61 mg AAE/g d.w., while the skins of the half-ripe fruit had the lowest TAC as 11.06 mg GAE/g d.w.

In DPPH assay, the investigated samples recorded the values ranged between 1.30 to 2.03 mg GAE/g d.w (Figure 4C). It was detected that skin, flesh pulp and the seeds of the fruit harvested 3 weeks earlier had the values of 1.30, 1.77 and 1.54 as mg GAE/g d.w, respectively for DPPH scavenging activity. The above-mentioned values significantly increased for fruit harvested on time to be 1.43, 2.03 and 1.72 mg GAE/g d.w, respectively.

Collectively, this study clarified that the antioxidant activity of kei apple different parts increases with the maturity advance when measured by TAC and DPPH assays, similar observations have been reported by Iordanescu et al. [39]. They examined the antioxidant potential of several apricot varieties at different ripening periods using the FRAP assay and concluded that full-ripe stage fruits had the highest antioxidant activity in comparison to those in half-ripe and green phases. To the best of our knowledge, no publications have previously described the antioxidant activity of kei apple different parts during maturity stages. Obtained data concerning TAC and DPPH assays demonstrating that the antioxidant potential of the fruit parts followed the following order: -flesh pulp > seeds > the skins. Likewise, the richness of TPs in kei apple different parts followed the former order indicating a potential relationship between TPs and the antioxidant activity. The higher antioxidant capacity of fruit pulp extracts in our study is quite agreed with the results obtained by Taher et al. [4] who found that whole fruit extract of kei apple had slightly higher antioxidant capacity than flesh pulp extract. This variation in the results of kei apple preparations might be explained by accession differences and/or environmental factors such as geography and the presence of biotic or abiotic stresses. Also, extraction method, solvents, particle size affect the composition of the extractable constituents. As shown in Figure (4A), the fruit pulp of kei apple fruit accumulated more level of phenolic constituents than other parts, the accumulation of phenolics and in particular 4HBA, might reflect the presence of biotic or abiotic stress [34-35, 40]. Likewise, the juice and the flesh pulp of kei apple fruit mostly contained higher contents of other non-enzymatic antioxidants like ascorbic acid, sugars and thiols [2, 4, 7]. In this respect, the impact of tert-hexadecane-thiol mostly existed in kei apple fruit pulp in improving the antioxidant activity has been reported [7]. Overall, the high antioxidant activity of kei apple whole fruit solvent extracts has been previously described [4-5, 7].

3.7 Relationships regarding the antioxidant activity

The correlation coefficients (R) and coefficients of determination (R^2) are displayed in Table 2. Linear correlation among each of both antioxidant assays was found with its correlation coefficient (r) of 0.923 for DPPH vs TAC. The correlations in between each assay of TAC and DPPH were compared with their TP subjecting for each of the correlation coefficients of 0.945 and 0.957. Results of R^2 (coefficients of determinations) for TAC (0.893), and DPPH

(0.9158) proposed that 89.30 and 91.58% of TAC and DPPH of the fruit, respectively, influenced by the concentrations of TP. Present study confirmed the reliability and the strong relationship of TPs on the antioxidant activity of kei apple fruit samples. Interestingly, a strong antioxidant activity of catechol using H_2O_2 and DPPH assays in comparison to other phenolic compounds and anilines has been previously stated [41]. Likewise, the antioxidant potential of monohydroxybenzoic acids against superoxide radical has been formerly reported [42]. The higher antioxidant capacity in the position para and ortho, makes 4-HBA as strong antioxidant as 2-HBA and far than 3-HBA. Collectively, the values of (R^2) are around ~90%, showing that main role of phenolic constituents in the fruit antioxidant capacity.

Table 2. Correlation results between TP, TAC, DPPH and K

	TP	TAC	DPPH
TP	1		
TAC	$r = 0.945^{***}$ (0.8930)	1	
DPPH	$r = 0.957^{***}$ (0.9158)	$r = 0.923^{***}$ (0.8519)	1
K	$r = 0.858^{***}$ (0.7361)	$r = 0.936^{***}$ (0.8760)	$r = 0.778^{***}$ (0.6052)

TP = total polyphenolic content; TAC = total antioxidant capacity; R, correlation coefficient. The values in parenthesis represent the R^2 *** significant level at $p < 0.0001$

Correlation among potassium with different total antioxidant capacity parameters was also studied (Table 2). A strong and positive relationship between K with TAC ($R = 0.936$, $p < 0.001$) could be detected. The potassium also exhibited a strong and positive relationship with DPPH ($R = 0.778$). In other words, the present study confirmed the relationship between K level and the obtained antioxidant capacity by TAC and DPPH. These findings largely agreed with those obtained by Tamuly et al. [43], who established a moderate correlation between K level and TPs, TF, ferric-reducing antioxidant power (FRAP), DPPH and 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)(ABTS) in underutilized vegetables. The mineral imbalance could affect the accumulation of antioxidant flavonoids [44]. The positive correlations between K level and TPs, DPPH and TAC in this study might be elucidated by the potential role of K in the activation of flavonoids and phenolic biosynthetic enzymes [45].

4. Conclusion

Data gotten in the present study, exhibit the nutritional quality of kei apple fruit cultivated in Egypt and its support of macro and essential mineral elements. Calcium and iron were largely accumulated into the skins of the kei apple fruit, while magnesium, copper and zinc were deposited into their seeds. The concentrations of some mineral elements were dramatically decreased or increased in kei apple different parts during the last step of maturation. The flesh pulp of fully ripe kei apple fruit displayed the highest antioxidant capacity and phenolic content, and the use of half-ripe fruit led to a decrease in all these parameters. Para-hydroxy benzoic acid and catechol were the main identified phenolic compounds in the seeds and the flesh pulp of ripening and ripe fruits. chlorogenic acid only found in the skin of the fruit.

Conflict of interest

The authors declare no competing financial interest.

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