

Chemical and Bioactive Composition in Persimmon (*Diospyros kaki*) Fruits

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ABSTRACT

Bioactive materials such as amino acids, organic acids, total phenols, vitamins, carotene and antioxidants of natural dietary fruits such as Persimmon kaki play an important role in human nutrition and health as potential sources of functional foods and nutraceuticals. Some of these components have beneficial effect on human health to their ability to prevent or control various diseases. The results showed that total sugars were 18.0%. Protein content in fresh persimmon fruits was 0.76%, fat 0.42%, fiber 3.5% and ash content was 2.35%. The total phenols were 298.01 mg. gallic acid per kilograms (kg). Persimmon fruits are rich in vitamin C (58.0 mg/ 100g), vitamin A (38.2 IU/ 100g). Persimmon fruits have many items of vitamin B, such as B1, B2, Thiamin, B6 and B9; its values are 97.5, 0.039, 10.5, 4.54 and 15.9 (mg/100g); respectively. Seven essential amino acids are presents in persimmon fruits; they were threonine, Isoleucine, Phenylalanine, Lysine, Methionine, Valine and Histidine. The flavonoid compounds that presented in abundance were Hesperidin, Quercetrin, Luteolin, Narengin and Rutin in decreasing order.

Keywords: Persimmon, Bioactive Composition, flavonoid compounds, vitamin

INTRODUCTION

The consumption of fruits and vegetables has become very important for the protection of health, because the presence of different bioactive components that show activity in the prevention of many Pathologic illness: There are two kinds of persimmon cultivated in Egypt; "astringent" Like Hachiya and kostata varieties and "non-astringent" like Fuyu and Hana fuyu. The first kind cannot eat right away because the astringent flavor which due to the high level of tannin (Kato, 1984) [1]; which classified the persimmon fruits to: fruits having 0.1-0.2% tannins are slightly astringent while those containing less 0.1% tannins are almost non- astringent and the fruits having more 0.2% tannins are highly astringent.

Bioactive materials such as amino acids, organic acids, total phenols, vitamins, carotene and antioxidants of natural dietary fruits i.e. Persimmon play an important role in human nutrition and health as

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potential sources of functional foods and nutraceuticals (Abeysinghe et al. 2007 and Jin Hwan lee et al. 2012) [2,3].

Although persimmon kaki is included among the minor fruits, it cannot be neglected as a food fruit given its chemical composition rich in substances not only of high biological value but also with therapeutic potential in the prevention of many pathologies. Among the fruits, Persimmon is a popular and widespread fruit that is enriched with many bioactive compounds, including polyphenols, terpenols, flavonoids, carotenoids, minerals and dietary fiber. Some of these components have beneficial effect on human health to their ability to prevent or control various diseases (Karaman et al., 2014 and Yaqub et al., 2013) [4,5].

The objective of this research were to determined physical characteries and chemical composition and bio-active such as, amino acids, phenolic components, fatty acids, vitamins and total antioxidants in the fruit of persimmons.

MATERIALS AND METHODS

Materials

Ripe mature seedless persimmon fruits (*Diospyros kaki* var. Fuyu) were purchased from a farm in Dakahlia Government; Egypt at the end of October 2022. The fruit had red-orange color, spherical shape with an average weight 150-170 gm. per fruit.

Methods

Pulp preparation: There is no need to remove the fruit skin; the ripe fruits were blended in food blender and strained through a sieve. Citric acid (0.1%) was added as a solution to the strained pulp to prevent darkening during thermal processing (Tonytantillo.com, 2001) [6].

Chemical analysis: Moisture content, protein, total carbohydrate, total sugar (reducing and non- reducing sugars). Dietary fiber and fat were determined according to A.O.A.C (2005) [7]. Ascorbic acid, PH, acidity and polyphenols as tannic acid were estimated according to Askar and Treptow (1993) [8]. Atomic absorption spectrophotometer Pye Unicam SP9 was used to determine minerals such as Na and K.

Total Phenol Content Analysis: The Folin-Ciocalteu method, based on the reduction of phosphowolframated-phosphomolybdate complex by phenolics to blue reaction products, was used to determinate phenolic compounds (Blahova et al; 2004) [9]. An aliquot of 10 µl of the extract was added to 250 µl of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water). The mixture was allowed to

stand for 3 min, then 2.5 ml of sodium carbonate Na₂CO₃ (7.5%, w/v) was added to the mixture. After 60 min in dark at room temperature, the absorbance of the solution was measured at 725 nm against a blank in a spectrophotometer (Analytik jena, SPECORD 200 PLUS). Results were expressed as gallic acid equivalents dry weight extract (mg GAE/g DW).

Total Antioxidant Determination: Performed by adding 3.5 ml of deionized water, 50µL of sample extract and Folin-Ciocalteu reagent and 300µL of sodium carbonate 20% to cuvette. The reaction was left for 15 minutes and then the absorbance was measured in triplicate at 730nm using a UV/VIS spectrophotometer (Shimadzu, Kyoto, Japan). The blank consisted of all reagents excluding the sample extract. A standard curve was fashioned using Tannic acid at concentrations of 0.2, 0.4, 0.6, 0.8, and 1.0mg/mL diluted in ethanol. Total phenolic concentration was expressed as mg of tannic acid equivalents via the standard curve (Singleton and Rossi, 1965) [10].

Determination of vitamin B group using HPLC: In brief, Sample (2 g) was placed in 25 mL of H₂SO₄ (0.1 N) solution and incubated for 30 min at 121°C. Then, the contents were cooled and adjusted to pH 4.5 with 2.5 M sodium acetate, and 50 mg Takadiastase enzyme was added. The preparation was stored at 35°C overnight. The mixture was then filtered through a Whatman No. 4 filter, and the filtrate was diluted with 50 mL of pure water and filtered again through a micropore filter (0.45 µm). Twenty microliters of the filtrate was injected into the HPLC system. Quantification of vitamin B content was accomplished by comparison to vitamin B standards.

Standard stock solutions for thiamine, riboflavin, niacin, pyridoxine, and cobalamin were prepared as reported previously.

Chromatographic separation was achieved on a reversed phase- (RP-) HPLC column (Agilent ZORBAX Eclipse Plus C18; 250 × 4.6 mm i.d., 5 µm) through the isocratic delivery mobile phase (A/B 33/67; A: MeOH, B: 0.023 M H₃PO₄, pH = 3.54) at a flow rate of 0.5 mL/min. Ultraviolet (UV) absorbance was recorded at 270 nm at room temperature.

Determination of Vitamin C using HPLC: The sample (10 g) was blended and homogenized with an extracting solution containing metaphosphoric acid (0.3 M) and acetic acid (1.4 M). The mixture was placed in a conical flask and agitated at 10,000 rpm for 15 min. The mixture was then filtered through a Whatman No. 4 filter, and samples were extracted in triplicate. The ascorbic acid standard

was prepared by dissolving 100 mg of L-ascorbic acid in a metaphosphoric acid (0.3 M)/acetic acid (1.4 M) solution at a final concentration of 0.1 mg/mL. The calibration line was converted to a linear range based on four measured concentration levels.

Quantification of ascorbic acid content was performed on an Agilent HPLC system. Chromatographic separation was achieved on an RP-HPLC column through isocratic delivery of a mobile phase (A/B 33/67; A: 0.1 M potassium acetate, pH = 4.9, B: acetonitrile: water [50:50] at a flow rate of 1 mL/min. UV absorbance was recorded at 254 nm at room temperature

Determination of Vitamin D3, A, E and Carotene: In 10 g sample, 1 g of pyro Gallic acid, 70 mL ethanol, and 30 mL (50%) KOH were added, stirred, and refluxed for 40 min using a water bath at °C [15,16]. Extracts were obtained three times using various ether concentrations (50 mL, 30 mL, and 20 mL). Double-distilled water was used to neutralize the extract, which was dehydrated using anhydrous sodium sulfate. Further, the extract was concentrated to approximately 5 mL by using a water bath (°C), diluted to 10 mL by using methanol, filtered using a 0.45 µm membrane, and finally subjected to HPLC analysis.

RP-HPLC analysis was performed with the Agilent 1100 series HPLC system (Agilent; USA), including a diode array detector.

The column was made of stainless steel. For -carotene quantification, the Agilent TC-C18 column was used (5 µm,

4.6 × 250 mm) with an acetonitrile-methyl alcohol-ethyl acetate (88:10:2) solvent, and UV absorbance was recorded at 453 nm.

For fat-soluble vitamins, the Agilent Eclipse XDB-C18 column was used (5 µm, 4.6 × 150 mm), the solvent was methanol, and UV detection was recorded at 325 nm for vitamin A, 265 nm for vitamin D3 and 290 nm for vitamin E.

Separation of all vitamins was based on isocratic elution and the solvent flow rate was maintained at 1 mL/min. Twenty microliters of okra oil was directly injected into the HPLC column. Fat-soluble vitamins were identified by comparing their retention times with those of authentic standards. All procedures were carried out under subdued light conditions.

Standard solutions of vitamins were prepared by serial dilution to concentrations of 0.1, 1, 5, and 10 mg per liter of vitamins D3, E, A, and -carotene, respectively. Standard solutions were prepared daily from a stock solution, which was stored in the dark at -20°C. Twenty microliters of standard solution was injected, and peak areas were determined to generate standard curves.

RESULTS AND DISCUSSION

Physical properties of persimmon fruits (*Diospyros kaki var fuyu*) are presented in Table 1. The number of fruits per kilogram is 7 fruits with mean average 143 gm. per fruit. The pulp percentage 91.4% and juice extraction was 90.0%. Total soluble solids (TSS) were 21.0% and PH value was 6.05. Visuable color of fruits ranged between the bright orange to red color.

Table 1: Physical Properties of Persimmon Kaki.

Number of fruits / Kg	7
Mean average / fruits (gm.)	143
Peels %	8.4
Pulp %	91.4
Juice extraction %	90
Total soluble solids (TSS)	21
PH	6.05
Visuable color	Bright orange to red color

Chemical composition of fresh persimmon fruits is listed in Table 2. It could be observed that moisture content was 73.47%, total carbohydrates was 18.9%, while total sugars was 18.0% with reducing sugars 2.27%, reducing sugars are representative more than 87.39% of total sugars. Protein

content in fresh persimmon fruits was 0.76%, fat 0.42%, fiber 3.5% and ash content was 2.35%. The total phenols were 298.01 mg. gallic acid per kilograms (kg). These results are in agreement with those obtained by Denev and Yordanov (2013) [11] and Homnava et al., (2014) [12]. The relative

contents of these compounds differ from author to author depending on the cultivar, organic conditions factors; such as light, temperature, humidity etc. (Novillo et al., 2015) [13].

Table 2: Chemical Composition of Persimmon Kaki (Fresh weight).

Items	Results (g/ 100gm)
Moisture	73.47
Total carbohydrate	18.9
Total sugars	18
Reducing sugars	15.73
Non – reducing sugars	2.27
Protein	0.76
Fat	0.42
Fiber	3.5
Ash	2.35
Total phenols (as gallic)	298.01 mg/ kg

Vitamin content in persimmon fruit are presented in Table 3. Persimmon fruits are rich in vitamin C (58.0 mg/ 100g), vitamin A (38.2 IU/ 100g). From this Table, it could be showed that persimmon fruits have many items of vitamin B, such as B1, B2, Thiamin, B6 and B9; its values are 97.5, 0.039, 10.5, 4.54 and 15.9 (mg/100g); respectively. Our results are slight difference with those obtained from review article by Yaqub et al., (2013). This may be attributed to the different variety of persimmon and / or agri environmental conditions. Meanwhile, these results are in accordance with

the results obtained by Thabit (2010) [14] and Gorinstein et al. (2011) [15]. Carotenoids in persimmon fruits given the specific color bright orange to reddish. α Carotene and β carotene content in persimmon fruits are illustrated in table 3. The fruits contained 83.0, 290.0 micro gram (mcg)/100g; respectively. While, β cryptoxanthin was not detected in fuyu variety which our research upon it. Forbus et al. (2011) [16] showed that, total carotenoids in persimmon fruits ranged between 212- 265 mcg/100g.

Table 3: Vitamin contents in persimmon kaki as dry weight.

Vitamins	Concentration	Units
Vitamin C.	58	mg / 100g
Vit. B1	97.5	mg / 100g
Riboflavin B2	0.039	mg / 100g
Thiamine	10.5	mg / 100g
Vit. B6	64.54	mg / 100g
Vit. B9	15.9	mg / 100g
Vitamin A	38.2	IU.
α carotene	83	mcg /100g
β carotene	290	mcg /100g

The quantification of amino acid plays an important role in knowing the nutritional quality of fruits and vegetables. Amino acid composition in persimmon fruits is presented

in Table (4). Fifteen amino acids are detected in persimmon fruits. Aspartic acid was the most abundant amino acid (83.0 mg/ 100g) followed by Valine (45.0 mg/100g).

Alanine content and threonine have (43 mg/100g) for each. Meanwhile, phenyl alanine, Arginine and Glycine were found in decreasing order. The lowest value was noticed in case in Isoleucine and lysine. Total amino acids were (375.0 mg/100g) of persimmon fruits. These results are in line with those obtained by Jin Hwan lee et al., (2012) [2] who reported that total amino acids in persimmon ranged between 187.72

to 390 mg/100g depending to the variety and location and may be also affected by environmental stress; Aspartic acid and glutamic acid were the highest concentration in all samples. The our results showed that have seven essential amino acids are presents in persimmon fruits; They were threonine, Isoleucine, Phenylalanine, Lysine, Methionine, Valine and Histidine.

Table 4: Amino acid composition in persimmon kaki (mg/100g, dry weight).

Amino acid	Results
Threonine *	43
Isoleucine *	0.03
Cysteine	16
Phenyl alanine *	39
Lysine *	0.04
Methionine *	2
Tyrosine	23
Valine *	45
Arginine	35
Histidine *	16
Alanine	43
Aspartic acid	83
Glutamic acid	18
Glycine	31
Proline	28
Total amino acid	375.0 mg/100g

*Essential amino acid

Phenolic and flavonoid compounds in fresh persimmons kaki are presented in Table (5). From this Table, it was eleven phenolic compounds; it were, gallic acid, pyrogallol, 4-amino-benzoic, tyrosol, chlorogenic acid, catechol, caffeine, caffeic acid, vanillic acid, ferulic and benzoic acids. Pyrogallol it was found in higher concentration (1834ppm) followed by catechol (83.37ppm) and benzoic acid (50.53ppm) in decreasing orders. Tyro sol recorded as the lowest value (3.3ppm) of the all detected phenolic compounds. Total phenolic compound was 216.5 mg/100gm of persimmon fruit. This results are different with findings of Veberic et al., 2010 [17]; Hudina et al., 2008 [18] and Oksuz et al., 2015 [19] due to the different studied varieties and agricultural factors.

Results presented in Table (5) also, showed the content of flavonoids compounds in persimmon fruit. Flavonoids including flavones and anthocyanin's are the most important to the protection against cancer, heart disease and their antioxidant effect. The flavonoid compounds that presented in abundance were, Hesperidin, Quercetrin, Luteolin, Narengin and Rutin in decreasing order; the values were 2698.6, 816.6, 559.4, 534.7 and 104.4 (μ /100g); respectively. Rosmarinic acid was the lowest value of flavonoids (47.3 μ /100g). Total flavonoids in persimmon fruits were (4818.7 μ /100g). These results were higher than those obtained by (Ksouri et al.,2009) [20]. Our results are compatible with Jin Hwan lee et al., (2012) [3].

Table 5: Phenolic and Flavonoid Compounds in Fresh Persimmons Kaki.

Phenolic compounds	Results (ppm)	Flavonoids	Results (μ / 100g)
Gallic acid	5.88	Luteolin	559.41
pyrogallol	1834.02	Narengin	534.70
4-Amino - benzoic	4.75	Rutin	104.44
Tyrosol	3.31	Rosmarinic	47.30
Chlorogenic acid	43.18	Hesperidin	2698.60
Catechol	83.37	Hispertin	57.61
Caffein	49.26	Quercetrin	816.63
Caffeic acid	43.71	Total	4818.65
Vanillic	28.84		
Ferulic	27.47		
Benzoic	50.53		
Total	2165.32	Total antioxidant	80.5%

CONCLUSION

Persimmon (*Diospyros kaki*) fruits are rich in vitamin C (58.0 mg/ 100g), vitamin A (38.2 IU/ 100g) and have many items of vitamin B, such as B1, B2, Thiamin, B6 and B9; its values are 97.5, 0.039, 10.5, 4.54 and 15.9 (mg/100g); respectively. Seven essential amino acids are presents in persimmon fruits; they were threonine, Isoleucine, Phenylalanine, Lysine, Methionine, Valine and Histidine. The flavonoid compounds that presented in abundance were Hesperidin, Quercetrin, Luteolin, Narengin and Rutin. All of these components have beneficial effect on human health to their ability to prevent or control various diseases.

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