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SOME CHEMICAL AND PHYSICAL PROPERTIES OF PERSIMMON FRUIT *(DIOSPYROS KAKI L.)*

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ABSTRACT

The problem at hand is aimed to shed light upon the chemical and physical properties of persimmon fruit cultivated under Egyptian environmental conditions. Other properties were concerned in terms of measuring the velocity of the enzyme pectin methylesterase and it's effect on the softening of persimmon flesh (without any addition of sugar solution) during storage for 90 days at -18°C. Identification of sugars in the investigated persimmon fruit was also considered by HPLC beside the changes in the pectin and vitamin "A" content. The chemical properties of the persimmon fruit include moisture (72.05 %), total soluble solids (20%), acidity as citric acid (0.160 %), ash (0.72%) and crude fiber (1.39 %). Vitamin "C" and "A" values recorded of 20.40 and 1.330 mg/ 100g fresh matter respectively. The level of vitamin "A" showed a proportional relation with storage period up to 90 days at –18°C. Experiments proved that unit of enzyme activity after 11 days in the sample stored at room temperature is approximately similar to the unit of activity scored after 90 days at –18°C. The corresponding units were found to be 45.467 mg CH_3O/100 ml sample and 42.37 mg CH_3O/100 ml juice.

Key words: Persimmon fruit, Carotene, Pectin methyl esterase, Polyphenols, Vitamins "A" and "C"

INTRODUCTION

Persimmon fruit *(Diospuros Kaki L.)* is now becoming increasingly one of the important export fruits. It's future success depends largely on the ability of the fruit to store well for two to three months at low temperatures without developing chilling injury; a trend which has been well documented by **MacRae (1987).** Chilling injury in its most severe form usually causes the fruit to be rubbery, appear mottled externally and the flesh firm and dark with a translucent gel form. It is therefore likely to describe that major changes are due to physicochemical changes of the cell wall that accompany chilling injury in persimmons, **(Grant *et al* 1992).** The latter authors also examined cell wall metabolism of persimmons

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during storage under different conditions and found that chilling-injured fruit passed through the normal ripening changes, being more rapid than those stored under normal temperature. In injured fruit, the solubilized polymers had a higher molecular mass and contained a higher proportion of neutral sugars than normally ripened fruit. Neutral sugars are usually associated with hemicelluloses, and they were lost from the insoluble cell wall material (CWM) in injured fruit but not in normally ripened fruit, and **(Woolf *et al* 1997)** proved that there was a net increase in the insoluble CWM during storage. However, fruit softening is thought to occur primarily as cell wall structure is modified during ripening, leading to a loss of tissue integrity.

The mechanisms of textural changes have focused on the degradation of cell wall pectin by polygalacturonase i.e., α-1,4-endo-D-galacturonase, **(Fischer and Bennett, 1991)**. A substantial evidence has also accumulated suggesting that other enzyme mechanisms are also involved in softening such as pectin methyl esterase, and cellulose.

Symptoms associated with chilling injury (CI) lead to a significant reduction in fruit firmness, off flavors, increased ethylene production on removal from storage beside major textural faults such as mealiness (loss of free juice) and in more severe cases, browning of flesh and skin, as well as formation of firm gel, **(Clark and MacFall, 1996)**.

Carotenoid type pigments, sugars and organic acids were estimated in persimmon fruits using HPLC under isocratic conditions. Carotenoids were identified as cis-mutatoxanthin, antheroxanthin, zeaxanthin, neolutein, cryptoxanthin, alpha-carotene and beta-carotene and fatty acid esters of cryptoxanthin and zeaxanthin. The fruits were an excellent source of retinol: 1 g provided about 54 IU of the vitamin. With respect to soluble sugars, the persimmon fruits contained mainly glucose, fructose and unidentified oligosaccharide, but no sucrose was detected in unripe or ripe fruits. Ion-pair HPLC allowed the separation and quantification of malic, isocitric, citric,ascorbic, fumaric and gallic acids, with malic acid being predominant. When fruits were fully ripe; metabolic processes led to a considerable loss in soluble sugars and organic acids; **(Daood *et al* 1992)**. Among other fruits; several researches propose persimmon as a good source of nutritional antioxidant, vitamins, polyphenols, and dietary fiber; **(Gorinstein *et al* 1994)**. Persimmon fruit has better tolerates among wide range of soil types and is less sensitive to nutrient deficiency. Production costs are significantly lower than citrus particularly for pruning, fertilizing and pesticide applications, **(Ko and Subhadrabandhu, 1997)**. Accordingly, this study was aimed to shed light upon the following points responded to persimmon fruit cultivated under Egyptian conditions:

- Chemical and physical properties of persimmon fruits produced under conditions predominating in Egypt;
- Effect of pectin methyl estrase on the flesh of persimmon fruit during storage at 25°C for 11 days and at 90 days at -18°C. The suitability of the fruit for producing juice is also of great attendance;
- Effect of storage on the pectin and vitamins "C" and "A" content of both whole fruit and the produced juice;
- Identification of sugars by HPLC.

MATERIAL AND METHODS

A: Materials

Mature and fully colored sweet persimmon *(Diospyros Kaki L.)* were purchased in October 2003 from El-Oboor market at Cairo governorate.

B: Methods

1- Technological treatments

About 40 kg of persimmon fruits were divided into three groups:

** The first group was stored in vented and perforated wholes carton boxes at 25°C for 11 days.

** The second group was kept in polyethylene bags and frozen in conventional freezer at –18°C (home style) and stored for 90 days without any addition of sugar solution. Such step was considered to see to what extent softening of persimmon could occur by the enzyme PME.

** The third group was pressed and the resulting juice was divided into two portions, one of which was stored for 90 days at –18°C and the other portion was fortified with 1% ascorbic acid and dried in a conventional air drier at 60°C overnight.

2- Analytical methods

Analyses of the investigated samples were performed at 15 day intervals.

2-a: Chemical analysis

2-a-1: Moisture, ash content, reducing and nonreducing sugars, fat, vitamins "A", and "C" as well as carotene were determined according to the **AOAC (2000).**

2-a-2: Fiber, pectin and acidity (as citric acid%) were determined by the method proposed by **Rangana, (1977)** and the results were calculated as g/100 g fresh sample.

2-a-3: Identification Sugars was carried out using HPLC according to **Daood *et al* (1992).**

2-a-4: Total phenols were determined by the method of **Gorinstein *et al* (1999)** and measured at 725 for crude polyphenols and 675 nm for total polyphenols.

2-a-5: Activity of PME: The procedure of **Fayyaz *et al.*, (1995)** was applied for measuring the activity of pectin methyl esterase in the presence of 1% pectin as recommended by **Abd Allah *et al* (2004).**

2-b: Physical analysis

2-b-1: Refractive index and pH value were measured by the method of the **AOAC, (2000).** For pH value, one gram of sample was blended in 25 ml distilled water for 2 min. and filtered through glass wool. The pH was determined by a model 610 pH (JENCO) electronica, LTD. USA, while refractive index was measured using a Carlzeiss Jena refractometer.

2-b-2: Water activity "a_w" or % ERH (Equilibrium Relative Humidity) of the dried persimmon juice was measured using a Rotronic Hygroskop DT as mentioned by **Cadden, (1988).**

2-b-3: Fruit index, weight, diameter, length and number of fruit were measured by **Kim and Ko (1995).**

3- Statistical analysis

Tests of linear and multiple regressions were applied according to SAS, **(1996)** and the level of significance is accepted as $P \geq 0.05$.

RESULTS AND DISCUSSION

I Major chemical constituents

The major chemical constituents of the persimmon fruit are given in Table (1) in terms of average of two replicates and calculated on fresh matter bases. Moisture content was 72.02% and the total sugars were 18.88%, of which reducing sugars were 13.59% representing 71.98% while nonreducing sugar that was 5.29% representing 28.02%. The total soluble solid was 19% and acidity (as citric acid) was 0.160%. Analysis also showed that persimmon fruits are characterized by a low level of fat (0.309/100g fresh sample) and ash (0.72%).

Crud protein and crude fiber were given in the same Table were 1.496 and 1.39 g/100 g fresh matter, respectively. Carotene showed a value of 0.303 g/100g fresh sample, while that of pectin was 4.873% and vitamin C recorded 20.40 mg/100g fresh sample. The aforementioned data coincide with those of **Ustun *et al* (1997)** who found that the chemical constituents of persimmons giving the following ranges: 17.04-20.70% dry matter, 14.0-18.9% soluble dry matter, 12.3-17.1% total sugar, 10.3-16.5% invert sugar, 0.38-1.90% sucrose, 0.06-0.14% titratable acidity, 10-18 formol number, 0.56-0.79% protein, 0.44-0.91% pectin, 0.42-0.53% ash, 6.8-19.65 mg/100g ascorbic acid, and 0.17-0.24 mg/100g total phenolic. **Grant *et al* (1992)** also found that persimmon fruits ripened after storage at 0° developed severe chilling injury and underwent many changes similar to those of the ripe fruits.

Table 1. Some chemical properties of the investigated Kaki fruit calculated g/100g fresh sample.

Constituent	Average of two replicates	Constituents	Average of two replicates
MOISTURE	72.02	Fat	0.300
Total sugar	18.88	Ash	0.720
Reducing sugar	13.59	Crud fiber	1.390
Non reducing sugar	5.29	Carotene	0.303
Total soluble solid	19.00	Pectin	4.873
Acidity (as citric acid %)	0.160	Vitamin C	20.40 mg/100g
Crude Protein	1.496		

These changes occur more rapidly and more extensive, and the solubilized pectic polymers that possessed a higher molecular weight (MW) contained a higher proportion of neutral sugars, than those solubilized during normal ripening. Glucose and xylose that normally associated with hemicellulosic polysaccharides were lost from the CWM of the chilling-injured fruits but not from normally ripened fruits.

Sugars content of the persimmon juice under investigation were identified by HPLC in the fresh and the dried form as seen in Table (2). The former contains 10.60% dextrose, 12.20% maltose, 77.20% maltytriose while those of the dried sample were: 25.739% fructose, 29.800% dextrose, 42.200% maltose and 0.937% maltytriose. Such dried persimmon juice contain 1.260% of high sugars. It seems evident that transformation of maltytriose took place during drying to other forms, i.e., maltose and glucose. It is of important to stress upon the fact that sugars of persimmons varied significantly with cultivar and maturity stage as given by **Senter *et al* (1991)** after using GLC and succeeded in identified arabinose, galactose, glucose, fructose and sucrose. The latter three sugars were predominant and present in all cultivars from mature green to fully ripe fruits.

Data in Table (3) showed some physical properties of the tested samples such as weight that recorded about 96.67g for each unit fruit sample with a diameter of 18.3 cm and 7.5 cm for length. It was also found that color appears to be ranging from dark orange to red and the number of fruits in one Kg was 11 units, while the fruit index (length/diam.) represents 0.41. In the opinion of **Kim and Ko, (1995)** such an index could be used to distinguish persimmons cultivars. The pH value (5.72) indicated that the sample was

Table 2. Sugars identification of fresh and dried persimmon juice samples.

Responded results	Identified Sugars				
	Fructose	Dextrose	Maltose	Maltytryose	High Sugar
Fresh Persimmon					
Retention time Sec.	-	10.66	8.00	7.20	-
Area cm^2	-	188116	215716	1370110	-
Concentration %	-	10.60	12.20	77.20	-
Dried Persimmon					
Retention time Sec.	13.31	10.64	9.55	6.30	17.44
Area cm^2	4513018	5232567	7402940	164291	220920
Concentration %	25.739	29.800	42.200	0.937	1.260

Table 3. Some physical properties of the investigated persimmon fruits.

Responded property	Value	Responded property	Value
Fruit weight (g)	96.67	pH	5.72
Diameter (cm)	18.30	Refractive Index	1.3640
Length (cm)	7.50	Color	Dark orange to red
Length / Diameter	0.410	Water activity	0.312
Number of fruits (kg)	11.00		

slightly non-acidic since fruits of pH more than 4.5 are considered nonacidic foods. However, the obtained pH is matched with **Ustun, (1997)** who reported a range of pH from 5.90 to 6.42 . On the other hand, refractive index was 1.3640 and water activity showed a level of 0.312 (% of equilibrium relative humidity) in the dried form of the persimmon sample. Such a low level of water activity prevents the reactivation of enzyme that may cause undesirable changes especially in persimmon flavor as it is well known that a value of water activity of 0.5 prevents the activity of enzymes.

The research of **Nam *et al* (1998)** indicated that sweet persimmons exhibited higher flesh pH than astringent persimmons. Differences in flesh pH within fruit types were not statistically significant. Overall results suggest that astringent persimmons had more attractive color appearance and contained more soluble sugars and organic acids than sweet persimmons.

The available data given in Table (4) showed that frozen persimmon fruit and the sample prepared in juice form had a carotene level of 3.092 mg/100 g juice sample measured at 436 nm at zero time of storage at -18°C. Such concentration increased to 5.827 mg/100 g persimmon juice after 90 days of storage at -18°C; thus, a real increment of 88.45% and a slope of 0.024 (Δ carotene/Δ day at –18°C) was recorded. In persimmon fruit carotene decreased to 1.489 mg/100g sample under similar storage conditions recording a decrement level of 51.84% with a slope of -0.029 (Δ carotene content /Δ storage period). The same trend was also noticed for vitamin "A" concentration; it's initial value that was 1.330 reached 2.565 in the persimmon juice increment level of 92.86% while dropped to 0.742 mg/100g in the whole persimmon fruit (decrement level of 44.21%) stored under similar conditions as seen in the same Table.

The bioactivity of the enzyme carotenase in the whole fruit and the usual physiological activities could play a serious role in explaining the decrement rate of carotene and vitamin A levels within a storage period of 90 days at -18°C. Such a trend could occur very rapidly during thawing before analysis **Leng *et al* (1993)** showed that, anthocyanin contents in cortical tissues of plants tended to increase when plants were exposed to temperatures of between -10° and -30°C. Also **Park and Kim (2002)** proved that carotene and lycopene contents increased by the end of storage period in drip of per-

simmon fruit. This pattern of changes could be related to the break down of other pigments associated with carotene especially chlorophyll with a possible increase in carotene value.

Homnava *et al* (1990) determined provitamin "A" (alpha-carotene, beta-carotene and beta-cryptoxanthin) and ascorbic acid in 14 cv. of Japanese persimmons *(Diospyros Kaki)* and 1 American persimmon. A remarkable variation in carotenoid content and carotenoid profiles was noted among cultivars. Provitamin "A" activity was found to be ranged from 17 retinol equivalents (RE) / 100g to 120 RE/100g. On the other hand, they assured that carotene was the predominant provitamin "A" isomer in 11 of 15 cv. with beta-cryptoxanthin predominant in the rest. Total ascorbic acid was ranged from 35 mg/100g to 218mg/100g. Mean concentrations of provitamin "A" and ascorbic acid were higher in nonastringent compared to astringent persimmons

Table 4. Changes in carotene and vitamin "A" concentrations (mg /100 g fresh matter) in persimmon (whole fruit and juice) during storage for 90 days at -18°C.

Storage period (days at–18°C)	Carotene concentrations				Vitamin "A"			
	juice		Whole fruit		juice		Whole fruit	
	OD (436 nm)	mg /100 g	OD (436 nm)	mg / 100 g	OD (450 nm)	mg / 100 g	OD (450 nm)	mg / 100 g
Zero	0.303	3.092	0.303	3.092	0.319	1.330	0.319	1.330
15	0.364	3.714	0.445	4.541	0.406	1.693	0.366	1.526
30	0.473	4.827	0.428	4.367	0.523	2.181	0.349	1.455
45	0.484	4.939	0.329	3.357	0.552	2.302	0.322	1.343
60	0.347	3.541	0.276	2.816	0.388	2.381	0.309	1.289
75	0.523	5.337	0.158	1.612	0.589	2.456	0.259	1.080
90	0.571	5.827	0.146	1.489	0.615	2.565	0.178	0.742
Intercept		3.3790		4.3482		1.5478		1.5545
Slope		0.0242		- 0.0291		0.0129		-0.0067
R^2		58.8 %		61.9 %		86.2 %		67.4 %
S.E		0.7188		0.8100		0.1834		0.1657
P		0.044		0.036		0.0025		0.0235
% of change		88.45		51.84		92.86		44.21

($p< 0.0001$). Subsequently, the Japanese persimmon is a good source of provitamin A activity and an excellent source of ascorbic acid.

It is of interest to shed light upon the crude and total polyphenols of the tested Persimmon juice and whole fruit (Table 5). The former one that measured at 725 nm indicated a decrement pattern with a slope of -5.5^{-5} Δ OD/ Δ day. A similar trend was noticed in the same sample for the total phenols measured at 675 nm giving a value of -2.5^{-4} Δ OD / Δ day. **Gorinstein *et al* (2001)** reported that the presence of total polyphenol in persimmon fruit beside its high contents of dietary fibers, main minerals and trace elements make persimmon fruit preferable for an antiatherosclerotic diet.

Experimental work was continued to find out and look forward to the activity of pectin methylesterase (PME) of the investigated persimmon samples in their whole and juice forms. Data in Table (6) and Figs (1 and 2) showed the activity of the enzyme PME within the tested sample from which unit of enzyme activity after 11 days in the sample stored at 25°C was approximately similar to unit of activity scored after 90 days at –18°C. For instance, the corresponding unit in case of whole persimmon was found to be 45.467 mg CH_3O/100 ml sample and 43.400 mg CH_3O/100 g sample.

Table 5. Changes in total polyphenoles in persimmon (whole fruit and juice) during storage at -18°C for 90 days.

Storage period in days at -18°C	Polyphenols concentration in juice as OD		Concentration of Polyphenols in whole fruit as OD	
	725 nm	675 nm	725 nm	675 nm
Zero	0.010	0.008	0.010	0.008
15	0.009	0.008	0.011	0.010
30	0.008	0.088	0.008	0.003
45	0.007	0.004	0.006	0.004
60	0.007	0.005	0.006	0.002
75	0.007	0.005	0.007	0.005
90	0.004	0.002	0.004	0.001
Intercept	0.00989	0.0286	0.0104	0.00814
Slope	-5.5^{-5}	-2.5^{-4}	-6.7^{-5}	-7.6^{-5}
R^2	0.87	0.069	0.784	0.5766

Table 6. Activity of PME in persimmon fruit (whole fruit and juice) during storage under different conditions.

Whole fruit stored at 25°C			Storage at -18°C				
Days	Average of 0.1 N NaOH	Activity unit	Days	Average of 0.1 N NaOH		Activity unit	
				Juice	Whole fruit	Juice	Whole fruit
Zero	0.157	1.622	Zero	0.157	0.157	1.622	1.622
1	3.850	39.783	15	3.725	4.200	38.492	43.400
2	3.425	35.392	30	3.275	3.925	33.842	40.560
3	3.650	37.717	45	2.150	3.325	22.220	34.360
4	4.435	45.823	60	3.325	4.350	34.360	44.950
5	3.425	35.392	75	3.900	3.825	40.300	39.525
6	4.025	41.591	90	4.100	4.200	42.370	43.400
8	4.425	45.725					
9	4.200	43.400					
11	4.400	45.467					

Unit Activity = mg CH3O \ 100 g sample.

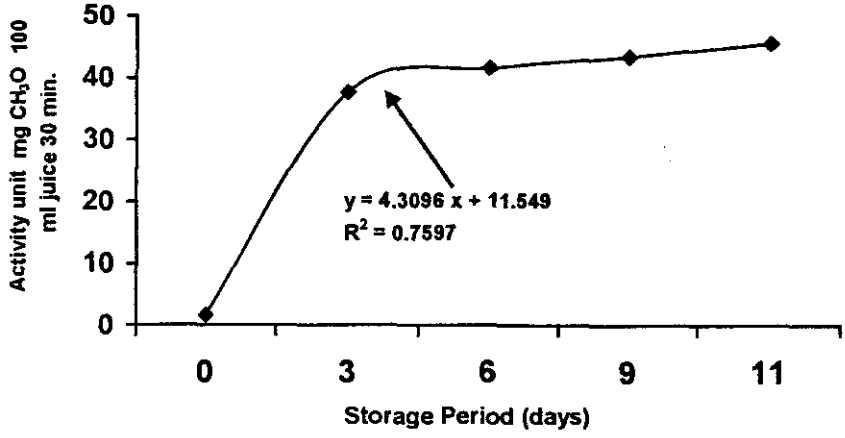

Fig. (1): Activty of PME in persimmon fruit held at 25°C for 11days.

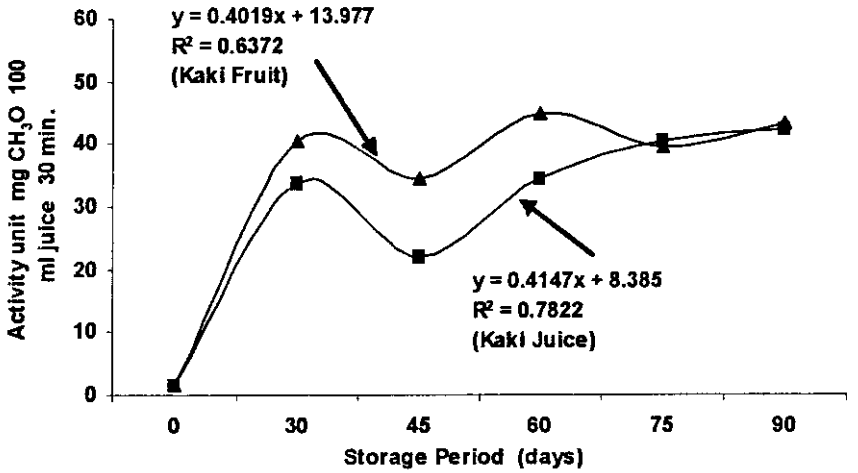

Fig. (2): Activty of PME in persimmon fruit and persimmon juice after 90 days of storage at -18°C.

Such a trend could be explained by the fact that the enzyme was found to be active within the thawing period before analysis. This result matched with the earlier findings of **Fishman and Oganesyan, (1984)** who stressed upon that the flesh of persimmon fruit had a highly active enzyme complex. Pectolytic activity decreased from 7956 units/g to 295 units/ g during storage for about 45 days (20 October to 5 December) andexopolygalactturonase activity decreased from 0.32 to 0.21 units/g, whereas pectinesterase activity increased from 0.07 to 0.18 units/g. During the first stage of enzyme activity; protopectin was hydrolysed, the flesh was softened followed by deep hydrolysis of pectin followed. In such a case, the fruit lost its astringency and acquired its characteristic flavor and aroma.

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بعض الخصائص الكيميائية والطبيعية المميزة لفاكهة الكاكى

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تم تقدير الخواص الكيميائية والطبيعية لفاكهة الكاكى مع الأخذ فى الإعتبار قياس سرعة نشاط إنزيم البكتين مثيل إستريز وتأثيرة على طراوة أنسجة الكاكى أثناء التخزين. ومن ناحية أخرى تم تصنيف أنواع السكريات فى نفس عينة فاكهة الكاكى المختبرة بواسطة جهاز HPLC بالإضافة إلى دراسة التغيرات فى محتوى البكتين وفيتامين A.

وتتضمن الخصائص الكيميائية الرطوبة (٧٢,٥%) والمواد الصلبة الذائبة (١٩%) والحموضة كحامض ستريك (٠,١٦٠%) والرماد (٠,٧٢%) والألياف الخام (١,٣٩%). كما سجلت نتائج فيتامين A ٢٠,٤٠- ١,٣٣٠ ملليجرام/ ١٠٠ جم مادة طازجة.

هذا وقد تبين أن مستوى فيتامين A أثناء التخزين لمدة ٩٠ يوماً على -١٨°م يتناقص نسبياً مع فترة التخزين. وقد أوضحت التجربة أن نشاط إنزيم البكتين مثيل إستيريز بعد ١١ يوماً من تخزين العينة على درجة حرارة الغرفة كان مساوياً لنشاط الإنزيم حال التخزين لمدة ٩٠ يوم على -١٨°م وكانت وحدة النشاط ٤٥,٤٦٧ ملليجرام CH_3O/100 ml و٤٢,٣٧ CH_3O/100 ml عصيراً.

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ا.د سعيد سلامة إبراهيم