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Influence of hot water and chitosan dipping and packaging methods on Storage ability of "Fuyu" and "Hachiya" Persimmon cvs.

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Abstract

The research was conducted in the refrigerated rooms of the College of Agriculture and Forestry / University of Mosul, and the experiment considered as a complete randomized design. Sound and similar fruits were selected and subjected to precooling, then, they were stored in the cold room at a temperature of $0\pm 1^{\circ}\text{C}$. The goal of this study is to study the influence of dipping persimmon fruit *Diospyros kaki* Thunb. cvs. "Fuyu" and "Hachiya" in 20 (Control), 40 and 50°C hot water, and 1% and 2% chitosan solution for 5 minutes, and packaged as vacuum polyethylene, or non-vacuum polyethylene packaging on fruits storability. After that, all treatment fruits stored for four months in cold room at 1°C and 80-90% RH. The results indicated that "Fuyu" fruits appeared a significant of fruit total sugars and total phenols than "Hachiya" fruits, while, "Hachiya" fruits produce a significant low respiration rate and POD enzyme. Chitosan or hot water dipping treatments maintained total phenols and fruit firmness, and suppressed respiration rate and PPO enzyme. Vacuum packaging method preserved fruit respiration rate. Interactions between the factors were effective in achieved fruit quality.

Keywords: chitosan, hot-water, non-vacuum, persimmon, packaging, vacuum.

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Introduction

Persimmon belongs to the genus *Diospyros* (family Ebenaceae). Around 400 species grow in ecological conditions ranging from tropical to more temperate climates. The species *Diospyros lotus* L., *Diospyros virginiana* L., and *Diospyros oleifera* Cheng have been used as rootstocks in several countries. These species are also utilized in medicine, pharmacy and in the cosmetic industry as a source of tannins. *Diospyros kaki* Thunb., which originated in China and is the only commercial species, includes cultivars whose fruits are consumed fresh or processed. Persimmons are deciduous trees that can be monoecious or dioecious and produce fruits parthenocarpically or via pollination. The fruit is generally oblong-conical, and varied in their size, shape and color. Fruit color could be yellow, orange or red of ripe fruits. In our country, the fruit normally harvested from late September to early December for the fresh market or storage. The fruits taste sweet; but many cultivars have an astringent taste until they are fully ripe. Persimmon classified into four types depending on the relationship between astringency, presence of seeds and flesh color. They are non-astringent only around the seeds and have dark tannin spots. Worldwide persimmon production is approximately 3 million MT. China, Korea and Japan account for 90% of total production; minor producers include Italy, Turkey, New Zealand and Spain. The major production areas are the Mediterranean, Black Sea and Aegean regions, where total production has reached 18,000 MT, about 80% of which comes from the Mediterranean region (Toplu et al., 2009). The treatment with Hot Water (HW) found to be effective as a physical method for the control of a wide range of storage pathogens or disorder and decay. Dipping many of fresh produce in hot water believed to improve the quality of fruit during a longed storage period. The possible reason of the effect of hot water in controlling and reducing fungal and disorder is the heat shock resulting from hot water treatment, which are able to induce host antifungal compounds that involved in resistance. In addition, hot water treatment of fruit at high temperatures was effective in controlling storage decay, by enhancing the resistance of fruit tissue, and slowing ripening. It is important to consider that hot water dipping treatment of fresh produce environmentally friendly, with out there no risk to human health. Besides, treatment with hot water would reduce production costs and consumer cost (Bakeer et al., 2016). In recent years, using edible coatings studied in order to preserve fruits and vegetables. Chitosan is a polymer edible coating, and consider one of the most promising biomaterials. In addition, the United States Food and Drug Administration has accepted chitosan as a food additive, and no symptoms reported in human's health. The most prevalent cationic polysaccharide is chitosan, which is a renewable resource and a low-cost biopolymer (Algarni et al., 2016).

Materials and Methods

Trees of persimmon fruits cvs. "Fuyu" and "Hachiya" chosen from a private orchard in Erbil /Kurdistan / Iraq to study influence of hot water and chitosan treatment, and vacuum packaging on the fruit storage ability of persimmon fruits during 2022 season. Mature "Fuyu" fruits harvested gently early of the morning on 27/October /2022, while "Hachiya" fruits harvested after 10 days. The fruits placed in plastic boxes and transferred to the cold room/ College of Agriculture and Forestry. Sound, uniform-size fruit chosen to perform the study. Fruits of persimmon cvs. "Fuyu" and "Hachiya" dipped in hot water at 20 (Control), 40 and 50°C or chitosan solution at 1 and 2% each for 5 minutes, and left to dry. Hot water and chitosan treatments of "Fuyu" and "Hachiya" fruits vacuumed electronically or not vacuumed, and placed in the cold room for four months at 1°C and 80-90% RH. The experiment considered as CRD Design, with three factors, chitosan and hot water and packaging method (2 x 5 x 2) with three replicates and by using six fruits for each treatment. The following traits measured:

Understudied traits

Fruit firmness (lb):

The firmness of the fruit flesh measured with a penetrometer of the type (Magness and Taylor pressure tester) (Kitinoja and Kader, 2002).

Total Sugar (TS %): measured as mentioned by (Joslyn,1970)

Respiratory rate (mg CO₂ Kg⁻¹ hr⁻¹):

The fruit respiration rate can be determined by following the quantitative method by (Gao et al., 1999). which involves measuring the quantity of carbon dioxide produced by one kilogram of fruit per hour, expressed as (mg CO₂ Kg⁻¹ hr⁻¹).

Total phenolic content in fruit (mg. g⁻¹ dry weight):

were estimated according to the method reported by (Gao et al., 1999).

by taking 0.25g of dried fruit ground plant leaves, and then 20 ml of ethanol was added to it and left for 24 hours and then placed in the electric vibrator for three times and for five minutes each time, and the sample was filtered and 0.1 ml of filtrate was taken and 0.2 ml of Volen reagent, 2 ml distilled water and 1 ml of sodium carbonate solution concentration of 15% until the color changes, after that the absorption measured by the spectrophotometer at a wave length of 765 nm by using the following equation

Peroxidase enzyme (POD) (Unit. minute⁻¹. g soft weight⁻¹):

The effectiveness of the enzyme peroxidase estimated according to the method described by Müftügil (1985). 2 ml of the reaction mixture consisting of 1 ml of H₂O₂ at a concentration of 1.0%, and 1 ml of Quaicol (dissolving 1.36 ml of Quaicol in a 250 ml volumetric flask, and then completing the volume using distilled water) was added to the spectrophotometer cell, after that 0.1 mL of the sample was added. The change in light absorption followed every 30 seconds for three minutes at a wave length of 420 nm. Then the number of enzyme units/ml calculated according to the following equation.

$$\text{Phenols (mg}^{-1}\text{-g dry weight)} = \frac{\text{Reading in the device} \times \text{Total extract volume} \times \text{Extract size used}}{\text{Sample weight} \times 100}$$

$$(\text{Number of enzyme units/ml}) = \frac{\Delta a \text{ Read Device} \times 3}{\Delta n \times 0.01}$$

Δ a: the change in light absorption.

Δ n: the duration of time taken for the change in absorption.

One unit of enzyme (the amount of enzyme that causes an increase in light absorption by 0.01 units per minute at a wave length of 420 nm).

Poly Phenol Oxides (PPO) (unit min⁻¹ g⁻¹ soft weight):

The effectiveness of polyphenol enzyme estimated according to the method described by Müftügil (1985) The effectiveness of Peroxidase was measured by adding (1 ml of 0.2 Müller potassium phosphate solution, and 7 PH and 1 ml of catecol solution (dissolving 0.248 g of catecol in a quantity of potassium phosphate buffrate solution and complete the volume to 100 ml and 1 ml of raw plant extract) in a spectrophotometer cell. The change in light absorption monitored every 30 seconds for 3 minutes at a wavelength of 420 nm. The results taken by recording readings of three replicates from each treatment. The activity of the enzyme calculated from the following equation:

$$(\text{Number of enzyme units/ml}) = \frac{\Delta a \text{ Read Device} \times 3}{\Delta n \times 0.01}$$

Δ a: change in light absorption.

Δ n: the duration of time taken for the change in absorption.

Results and Discussion

Firmness (lb.): Firmness did not differ significantly between the two cvs. The higher firmness resulted from fruits dipped in 50°C hot water and 1% and 2% chitosan, and differed significantly than the lowest firmness of control fruits (Table 1). The firmness loss of fruits happened by the degradation of insoluble pectin's to more soluble pectin's and to soluble pectic acids. This caused by the activities of pectin esterase and polygalacturonate, which produce the shortening of chain length of the pectin. Besides, chitosan coating application delays the firmness loss rate due to a hydrostatic pressure increasing and the respiration rate decreasing (table 1) (Blum et al., 2009). Chitosan act as barrier and do not allow oxygen and other substances to pass through it (Joslyn, 1970.) It found that coatings apricot fruits surface with chitosan succeed to reduce oxygen permeability, and reduce respiration rate. Gradually, ripening process and hydrolysis activities would slow down, which can cause fruit softening. These activities shown how chitosan coatings preserve fruit firmness (Lacroix and Le Tien 2005). Vacuum packaging fruits were more firmly significantly than non-vacuumed packaging fruits. Dipping of non-vacuumed packaging "Fuyu" fruits in 50°C hot water recorded the highest fruit firmness, which differed significantly from many interaction treatments (Table 1), on other hand, control vacuumed packaging "Hachiya" fruits gave the lowest firmness.

Table (1.) Effect of hot water (HW) and chitosan dipping, and packaging method on Firmness (lb.) of "Fuyu" and "Hachiya" fruits stored for four months.

cvs.	Treats.	Vacuum pack.	Non-vacuum Pack.	cvs. Mean	treats. mean
"Fuyu"	Control	9.33 ab	8.66 b	10.93 a	
	40°C (HW)	11.33 ab	8.66 b		8.75 b
	50°C (HW)	13.66 ab	15.33 a		10.33 ab
	1 % Chitosan	13.00 ab	9.66 ab		11.91 a
	2 % Chitosan	11.33 ab	8.33 b		11.33 a
"Hachiya"	Control	8.00 b	9.00 b	10.46 a	11.16 a
	40°C (HW)	10.33 ab	11.00 ab		
	50°C (HW)	9.33 ab	9.33 ab		
	1 % Chitosan	10.00 ab	12.66 ab		
	2 % Chitosan	13.00 ab	12.00 ab		
Pack. Mean		10.93 a	10.46 b		

Total sugars (%): Significant differences appeared in the total sugars, and were the highest in the fruits of the "Fuyu" cv. Total sugar in all treatments was not significantly different, although the highest value was when the fruits dipped in 2% Chitosan (Table 2). Chen et al., (2016) Stated that edible coating could increase total sugar in mandarin fruits, may be because coatings provided a beneficial film around the fruits, that modifying the internal atmosphere by elevating CO₂ and reducing O₂ levels. This phenomenon found to delay the degradation rate of nutrients in fruits (Lin and Zhao, 2007). On other hand no significant differences shown in total sugars, whether the fruits packaged in vacuum or non-vacuum packs (Table 2). In general, significant differences appeared between the two varieties and in all transactions, whether vacuum or non-vacuum, and the highest values of total sugars

were recorded "Fuyu" cv. fruits, which were dipped in 2% Chitosan and were vacuumed.

Table (2). Effect of hot water (HW) and chitosan dipping and packaging method on total sugars (%) of "Fuyu" and "Hachiya" fruits stored for four months.

cvs.	Treats.	Vacuum pack.	Non-vacuum Pack.	cvs. mean	treats. Mean
"Fuyu"	Control	21.19 ab	22.34 ab	21.53 a	17.41 a
	40°C (HW)	20.93 abc	21.88 ab		18.21 a
	50°C (HW)	20.42 abc	20.80 abc		17.04 a
	1 % Chitosan	20.60 abc	21.37 ab		17.43 a
	2 % Chitosan	23.86 a	21.90 ab		19.31 a
"Hachiya"	Control	12.36 d	13.76 d	14.23 b	
	40°C (HW)	17.32 bcd	12.70 d		
	50°C (HW)	13.51 d	13.41 d		
	1 % Chitosan	14.37 d	13.38 d		
	2 % Chitosan	15.19 cd	16.30 bcd		
Pack. Mean		17.97 a	17.97 a		

Respiration rate ($\text{mg CO}_2 \text{ kg}^{-1} \text{ hr}^{-1}$): lower significant respiration rate observed in "Hachiya" fruits compared to "Fuyu" fruits. May be because the higher sugars content of "Fuyu" cv. fruits, as sugars is the preferable substrate for respiration, also, chitosan or hot water treatment inhibited respiration rate compared to control treatment. The lower respiration rate in chitosan persimmon treated fruit might be attributed to the effect of chitosan as gas barrier, and similar result reported by (Uliana et al., 2014) while no significant differences appeared between packaging methods (table 3). It noticed from table that control treatment of vacuumed or non-vacuumed "Fuyu" fruits or "Hachiya" fruits appeared the highest respiration rate and differed significantly from other interaction treatments.

Table (3). Effect of hot water (HW) and chitosan dipping, and packaging method on respiration rate of "Fuyu" and "Hachiya" fruits stored for four months.

cvs.	Treats.	Vacuum pack.	Non-vacuum Pack.	cvs. mean	treats. mean
"Fuyu"	Control	11.66 a	11.00 a	8.60 a	
	40°C (HW)	7.33 de	7.33 de		8.75 b
	50°C (HW)	7.66 de	7.33 de		10.33 ab
	1 % Chitosan	8.33 cde	8.33 cde		11.91 a
	2 % Chitosan	8.66 cd	8.33 cde		11.33 a
"Hachiya"	Control	9.66 bc	10.66 ab	7.83 b	11.16 a
	40°C (HW)	7.33 de	7.33 de		
	50°C (HW)	7.33 de	7.00 e		
	1 % Chitosan	7.33 de	7.66 de		
	2 % Chitosan	7.00 e	7.00 e		

Pack. mean	8.23 a	8.23 a
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Phenols: no significant differences appeared between the two persimmon cultivars (Table 4), and the highest phenol was in persimmons that dipped in 2% Chitosan, while the least was from control fruits. The fruits treated with chitosan (2%) produce maximum total antioxidant activity. These results are in agreement with earlier reports, with strawberry fruits treated with chitosan during cold storage (Petriccione et al. 2015), and agreed with previous documents that chitosan treatment improved fruit properties by maintaining high levels of phenols during postharvest treatments (Wang and Gao 2013). Chitosan controlled the decreasing phenolic content activity of mango fruits compared to control (Toor and Savage, 2005). No significant differences appeared of phenol between packaging methods. In most of the dipped and packaging methods treatments of the both cvs. fruits, significant differences appeared, and the "Fuyu" fruits that dipped in 1% Chitosan and vacuumed achieved the highest phenol content, on the contrary, the lowest value was from control vacuumed "Hachiya" fruits (Table 4).

Table (4). Effect of hot water (HW) and chitosan dipping, and packaging method on Phenols of "Fuyu" and "Hachiya" fruits stored for four months.

cvs.	Treats.	Vacuum pack.	Non-vacuum Pack.	cvs. mean	treats. mean
"Fuyu"	Control	2.15 cd	2.93 cd	4.061 a	2.120 c
	40°C (HW)	2.77 cd	3.58 bcd		2.461 bc
	50°C (HW)	3.45 bcd	6.80 a		3.540 ab
	1 % Chitosan	6.88 a	1.98 cd		3.224 abc
	2 % Chitosan	5.73 ab	4.30 bc		3.672 a
"Hachiya"	Control	1.44 d	1.94 cd	1.94 b	
	40°C (HW)	1.86 cd	1.63 d		
	50°C (HW)	1.67 cd	2.22 cd		
	1 % Chitosan	1.84 cd	2.19 cd		
	2 % Chitosan	1.81 cd	2.82 cd		
Pack. mean		2.964 a	3.043 a		

Peroxidase (POD): The two cvs did not differ significantly of POD contents (Table 5). All dipping treatments were superior significantly of POD contents on control treatments except 1% chitosan treatment. The delay of chitosan coatings of the changes of antioxidant activities with during storage and ripening reported by Ali, et al., (2013). Edible coatings could delay the biochemical and physiological changes in fruits during storage. The antioxidant capacities of persimmon fruits depend on other factors like genetic factors, environmental conditions, cultivar and harvesting maturity (Ferreira, et al., 2012). While, no significant differences appeared of POD contents between packaging methods. On other hand, the highest POD contents obtained from "Fuyu" cv. fruits dipped in 50°C hot water and not vacuum packaged and differed significantly from control "Fuyu" and "Hachiya" cv. fruits which were the lowest (Table 5), and no significant differences appeared from the other interaction treatments.

Table (5). Effect of hot water (HW) and chitosan dipping, and packaging method on POD (%) of "Fuyu" and "Hachiya" fruits stored for four months.

cvs.	Treats.	Vacuum pack.	Non-vacuum Pack.	cvs. Mean	treats. mean
"Fuyu"	Control	78.00 ab	67.73 b	104.87 a	77.81 a
	40°C (HW)	136.11 ab	103.11 ab		109.23 a
	50°C (HW)	115.66 ab	159.70 a		108.27 a
	1 % Chitosan	83.07 ab	86.08 ab		84.59 a
	2 % Chitosan	90.76 ab	128.45 ab		110.87 a
"Hachiya"	Control	98.30 ab	67.19 b	16.76 b	
	40°C (HW)	98.16 ab	99.53 ab		
	50°C (HW)	86.01 ab	71.71 b ab		
	1 % Chitosan	78.96 ab	90.24 ab		
	2 % Chitosan	95.30 ab	128.96 ab		
Pack. Mean		96.03 a	100.27 a		

Poly Phenol Oxidase (PPO): PPO activity may consider a major browning enzyme, which resulted from the oxidation of phenolic substrates, which resulted in drop of fruit quality. It was most possibly that coating treatment decreases the activity of poly phenol oxidase enzyme in response to modifications in the internal atmosphere of coated fruit (CO₂ and O₂ rate) (Varasteh et al., 2012). Dong et al. (2004) also stated that application of chitosan coating treatment reduced enzyme activity which degradation of anthocyanin in fruit caused by poly phenol oxidase, over storage period. Sathiyabama et al., (2019). Reported that chitosan could prevent increasing of PPO activity by preserving cell membrane stability, and activation of antioxidant enzymes and genes related to ROS scavengers in treated fruit.

The two cvs. did not differed significantly of PPO contents (Table 6). Clearly, significant differences appeared between the highest contents of PPO of the control fruits and all the dipped treatments fruits except 1% chitosan treatment fruits and in both methods of packaging, no significant differences appeared. Significant differences appeared in all the fruits dipped for both varieties and vacuum, non-vacuum so the highest content PPO noticed in the fruits of control and vacuum in "Fuyu" cvs. ruits (Table 6).

Table (6). Effect of hot water (HW) and chitosan dipping, and packaging method on PPO of "Fuyu" and "Hachiya" fruits stored for four months.

cvs.	Treats.	Vacuum pack.	Non-vacuum Pack.	cvs. mean	treats. mean
"Fuyu"	Control	0.263 ab	0.244 abc	0.219a	0.265 a
	40°C (HW)	0.267 ab	0.124 ed		0.188 b
	50°C (HW)	0.219 a-e	0.226 a-d		0.199 b
	1 % Chitosan	0.259 ab	0.180 b-e		0.235 ab
	2 % Chitosan	0.141 cde	0.269 ab		0.209 b
"Hachiya"	Control	0.295 a	0.259 ab	0.219a	
	40°C (HW)	0.114 e	0.248 abc		
	50°C (HW)	0.224 a-e	0.126 ed		
	1 % Chitosan	0.251 abc	0.251 abc		
	2 % Chitosan	0.213 a-e	0.215 a-e		
Pack. Mean		0.224 a	0.214 a		

Conclusion: From the last illustrated results, we can conclude that the treatments of chitosan and hot water proved to improve the studied traits (firmness, respiration, phenols and peroxidase enzyme), and reduce poly phenol oxide enzyme, that can maintain fruit quality, so

we succeed to prolong persimmon fruits storage safely up to 4 months.

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