Effect of harvesting date and immersion treatment on the specific traits for local sweet lemon during cold storage

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ABSTRACT

The experiment was conducted in the Laboratory of Postgraduate Studies belonging to the Department of Horticulture and Landscape Gardening, College of Agriculture, Al-Qasim Green University for the season of 2017 on the fruits of the local sweet lemon obtained from one of the private orchards in Baqubah, Diyala province from trees with 30 year age cultivated under the palms to study the effect of harvesting date and immersion in calcium chloride solutions at a concentration of (1, 2 $g.L^{-1}$) for 5 min, Chitosan at a concentration of (20, 20 $g.L^{-1}$) for 10 min and salicylic acid at a concentration of (0.4, 0.8 $g.L^{-1}$) for 10 min on the traits of the stored fruits for three months at a temperature of (5 ± 1 °C) and relative humidity (85 - 90%). A factorial experiment was conducted with two factors according to the completely randomized design (CRD), with three replicates. The differences between the averages were compared according to the least significant difference test (L.S.D) at the probability level of (0.05), the results can be summarized as follows:

1- The fruits of the second date (December) were significantly excelled by reducing the rate of fruit respiration compared to the fruits of the first date (November).
2- The use of Chitosan at a concentration of (10 $g.L^{-1}$) led to increasing the percentage of juice and the juice/peel ratio. While the same treatment was characterized by reducing the percentage of the fruit's peel to 31.07% compared to the fruits treated with salicylic acid at a concentration of (0.8 $g.L^{-1}$), which maintained the highest percentage of the fruit's peel amounted to (42.71%). It was also characterized by reducing the percentage of spoilage to 4.75% compared to non-treated fruits in which the spoilage rate was raised to (11.92%), While the fruits treated with Chitosan at a concentration of (20 $g.L^{-1}$) were characterized a significant decrease in the average of respiration speed which amounted to (5.423 mg CO_2 / kg/hr) compared with untreated fruits in which the average increased to (5.699 mg CO_2 / kg/hr).

1. INTRODUCTION

Local sweet lemon (*Citrus limetta* L.) belongs to the Rutaceae family. It is located in the group of Indian lemons, and its native habitat is in northeast India, which is called (Misha Nimbu). Its growth is widespread in India, Pakistan, Iran, and Egypt (1, 2). According to the latest statistics, sweet lemon production in Iraq amounts to (721 tons) for the winter season 2018. As for the average production per tree, it is estimated at (12.0 kg) (3). In general, citrus fruits are characterized by their high nutritional value where it is considered a major source of vitamin C in addition to vitamin B12, B2, B1, A, and some nutrients and organic acids, such as Citric acid, amino acids and sugars (4). Citrus fruits are exposed to many pathogens and mechanical and physiological damage, starting from the harvesting process and passing through the operations of transport, circulation, and marketing, which negatively reflects on their quality and storability, Thus, its damage and the occurrence of large economic losses. Therefore, to reduce those losses, and to enhance the natural resistance of the fruits and maintain their quality after harvest and during storage, environmentally friendly and safe techniques were used, either for the fruit or the consumer, including treating with calcium compounds, since it is an essential element in strengthening the quality of fruits through the stability of cellular membranes and delaying Senescence in Horticultural crops where submerging the fruits after harvesting with a calcium solution increases their calcium content significantly compared to spraying before

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harvesting. It also works to support cell swelling, cell membrane consistency, tissue stiffness, delaying the demolition of lipids membrane and prolonging the shelf life of the fruits (5). Chitosan can also be used, which serves as envelopes, Preservative coatings, edible and biologically safe for different types of fruits due to its functional advantages such as reducing respiratory rates, prolonging the storage periods and controlling microbial growth (6, 7). Salicylic acid is a plant hormone that recent research has sought to study its effectiveness and effects on biological and physiological processes in plant growth, flowering, and absorption of ions (8). Its use has shown that it has a high ability to delay ripening, enhancing the quality and controlling post-harvest losses of fruits and vegetables (9). The researcher (10) found that harvesting the local orange fruits in January led to a significant decrease in the percentage of infection with physiological damage and reducing the respiratory speed rate as well as a significant increase in the percentage of juice, while the fruits obtained in December were characterized by a significant decrease in the percentage of microbial damage, the harvesting date did not have a significant effect on the percentage of weight loss after 90 days of cold storage. The researcher (11) also found that harvesting the fruits of Washington navel orange in December caused a significant decrease in the traits of losing weight with the increase in storage periods. In a study conducted by (12) on the Eriobotrya fruit (Surkh cultivar) stored at 4 ˚C for a period of 10 weeks, he observed that treating fruits with calcium chloride at a concentration of 3% led to a reduction in weight loss in the fruits, Al-Shammari and Marwa, (13) observed that treating apricot fruits (Zagainaa) with calcium chloride at a concentration of 5% led to a reduction in the percentage of weight loss, the percentage of spoilage and the respiration rate in the fruits. Mahfuzah et al., (14) found that treating strawberry fruits with calcium chloride at a concentration of (1%) led to a reduction in the percentage of weight loss which amounted to (0.123%) compared to untreated fruits. During the cold storage of Litchi fruit, (15, 16) observed a decrease in respiratory rate when treating fruits with Chitosan solution, Milena et al., (17) found when studying three cultivar of strawberry fruits and storing them for a period of 9 days at a temperature of 2 ° C, it was found that the fruits coated with Chitosan at a concentration of 2% led to a reduction in weight loss which amounted to (0.49%, 0.53, 0.59) compared to the non-coated fruits which amounted to (6.97%, 8.40%, 8.98%) respectively. He also indicated that treating with Chitosan has worked to delaying qualitative changes in color, it also prevents the appearance of brown color and reducing membrane decay and slowing the rate of deterioration compared to the fruits not covered in the tested cultivars. Zahoorullah et al., (18) observed when studying the banana fruits that the percentage of weight loss decreased to (4.5%) when treating the fruits with Chitosan. In a study conducted by (19) on the stored orange fruits for a period of 93 days at a temperature of 5 ° C, he noted that treating fruits with salicylic acid at a concentration of (1.1 $mg.L^{-1}$) led to a decrease in the percentage of fruit decay from 16.93% to 6.06%, and this concentration contributed to reducing Cold harms and preserving the quality of the fruits compared to the lower concentrations and untreated fruits. Kazemi et al, (20) also indicated that treating Kiwifruits with salicylic acid led to a decrease in weight loss and the percentage of decay to the fruits. Barman, (21) observed that treating mango fruits with salicylic acid at a concentration of (2 mmol) led to a decrease in the weight loss and softening of the fruits.

2. MATERIALS AND METHODS

The experiment was conducted in the Laboratory of Postgraduate Studies belonging to the Department of Horticulture and Landscape Gardening, College of Agriculture, Al-Qasim Green University for the season of 2017 on the fruits of the local sweet lemon obtained from one of the private orchards in

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Baqubah, Diyala province from trees with 30 year age cultivated under the palms. Fruits harvested with two dates when they were in commercial (mathematical) ripening. First date (D1) in mid-November and second date (D2) in mid-December. The healthy and homogeneous fruits were chosen in terms of size and color, and the fruits were divided into three groups. The fruits of the first group was immersed in calcium chloride solutions at a concentration of (1, 2 $g.L^{-1}$) for 5 min which is symbolized by (T5, T4), The fruits of the second group was immersed in chitosan at a concentration of (20, 20 $g.L^{-1}$) for 10 min which is symbolized by (T3, T2) and The fruits of the third group was immersed in salicylic acid at a concentration of (0.4, 0.8 $g.L^{-1}$) for 10 min which is symbolized by (T7, T6) In addition to the control fruits (without immersion) which is symbolized by (T1). After the fruits dried, they were placed in 1 kg plastic bags, perforated with eight holes on each side, and stored for three months at a temperature of (5 ± 1 °C) and relative humidity (85 - 90%). A factorial experiment was conducted according to the completely randomized design (CRD), with two factors, the first factor represents the harvesting date D, and the second factor represents the imprison solutions T. Each concentration included three replicates, with a rate of five fruits per replicate. The data was analyzed using the Genstat statistical program. The differences between the averages were compared according to the least significant difference test (L.S.D) at the probability level of (0.05) (22).

The studied traits:

The traits were studied periodically during the storage period by taking three fruits randomly from each treatment as follows:

1- The percentage of weight loss

The percentage of weight loss =

$$\frac{\text{The fruits weight after a certain period} - \text{the weight of the fruits in beginning storage}}{\text{the weight of the fruits in beginning storage}} \times 100$$

2- percentage of Juice

It was calculated according to the following formula:

$$\text{Percentage of fruit juice} = \frac{\text{weight of juice}}{\text{fruit weight}} \times 100$$

3- percentage of Fruit peel

It is calculated according to the following formula: -

The percentage of the fruit peel = (the peel weight) / (the fruit weight) x 100

4- Juice/peel ratio

5- Respiratory rate

The respiration rate was estimated using the Closed system method according to (Al-Ani, 1985) and it was estimated according to the following equation:

$$\text{mg } CO_2\text{/ Kg/ hr} = \text{mg } CO_2 \times \frac{1}{hr.} \times \frac{1}{Wt.(kg)}$$

where: Wt. = Weight of the sample in kilograms

hr. = The number of hours

6- Percentage of damage

The fruits were considered damaged as soon as any pathological or physiological injury appeared for the last period of storage only and it was calculated as in the following formula: -

$$\text{Percentage of damage} = \frac{\text{weight of damaged fruits}}{\text{total weight for treatment}} \times 100$$

3. RESULTS AND DISCUSSION

1- The percentage of Weight loss (%)

Weight loss is the most important factor affecting the storing traits for the fruits, where its exposure to inappropriate conditions, whether after harvesting or during storage, will lead to weight loss, which reduces their specific value, thus reducing their storability and marketability and reducing resistance to infection with pathogens (23, 24). Table (1) did

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not show significant differences between the two harvesting dates in the percentage of weight loss despite its decrease in the second date (D2) to 10.95 compared to the first date (D1) which amounted to (12.00), and the type of solution did not have a significant effect on this percentage. The values of bi-interaction between the harvesting date and the treatments indicated that this trait was affected significantly, where the D2T4 treatment showed significant superiority in reducing this trait to (8.17) while the D2T6 treatment increased the percentage of weight loss to amount to (15.92). Most studies indicate that there are many factors that can directly or indirectly affect the rate of moisture loss that occurs for fruits after harvest and during storage, including the ripening stage and the harvesting date (25). The storage conditions, in particular, the temperature and relative humidity play a large role in the amount of Weight loss as well as stored size and type of used packaging (26). The importance of calcium comes in this aspect as it is one of the most important compounds that enter the membranous system for the plant cell (27) as well as its great role in improving the construction of cell walls, which is included in the composition of the primary wall and the middle plate that works to link the adjacent cells in the form of calcium pectates (28, 29). The presence of calcium in this way will reduce the degradation of the cell wall by decomposition enzymes such as Pectinase. It also collects the crosslinked units of Pectic acid in the cell wall, which makes it more durable (30).

Table 1: Effect of harvesting date, immersion treatment and their interaction on the percentage of weight loss for local sweet lemon fruits.

Harvesting date	Immersion treatment						The average effect of harvesting date
	T1	T2	T3	T4	T5	T6	
D1	12.43	12.05	13.79	11.54	10.16	11.30	12.00
D2	11.51	11.78	9.82	8.17	9.48	15.92	10.95
Average effect of treatments	11.97	11.92	11.80	9.86	9.82	13.61	D= N.S
L.S.D 0.05	T= N.S			D×T= 6.888			

2- The percentage of Juice in fruits (%)

The harvesting date had no significant effect on the percentage of juice despite the noticeable increase in its averages on the second date (D2). As for the effect of the type of solution, the treating with Chitosan at a concentration of (10 $g.L^{-1}$) increased the average of this trait in fruits to 53.16%, with a significant difference from the control treatment T1, which gave the lowest average for this trait of (38.02%). It is also noticed that the interaction between the harvesting date and the type of solution affected significantly in this trait, where the D2T2 treatment has excelled by increasing it to (53.74%) while the D1T1 treatment was a cause to decreasing it to 34.34%. The first date also showed a noticeable decrease in the percentage of the fruit peel, which amounted to (36.41%) at the end of the storage period, but it did not differ significantly from the second date (D2), which recorded the highest average for this trait amounted to (36.44%). As for the type of solution, the treating with salicylic acid at a concentration of (0.8 $g.L^{-1}$) caused a significant increase in this trait amounted to (42.71%), while it decreased to 31.07% in the fruits treated with Chitosan at a concentration of (10 $g.L^{-1}$). As for the interaction treatment between the harvesting date and the type of solution, where the D2T7 treatment has excelled in increasing this trait which amounted to (42.81%), while the D1T2 treatment led to decreasing it to (30.97%). It is also evident that no significant differences appeared between the two harvesting dates in the juice/peel ratio, while the type of solution was significantly

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affected. The treating with Chitosan solution at a concentration of (10 $g.L^{-1}$) achieved significant differences in the juice/peel ratio for sweet lemon fruits, which recorded the highest average for this trait amounted to (1.733%), with a significant difference from the fruits treated with calcium chloride (2 $g.L^{-1}$) that gave the lowest average for this amounted to (1.152%). Significant differences also appeared between the values of bi-interactions, where the D2T2 treatment gave the highest juice/peel ratio for sweet lemon fruits amounted to (1.746%) compared to the lowest average for this trait recorded at the D1T7 transaction which amounted to (1.127%). It is noted that the factors that led to a rising percentage of weight loss in the fruits indirectly affected the increase in the percentage of juice and the decrease in the percentage of the peel as shown in Table (2), where the waxy material that encapsulates the fruits, works to form a pivoting envelope covering the fruits, it works to reduce the gas exchange and prevents the respiration rate from increasing, thus delaying the ripening and Senescence of the fruits (31).

Table 2: Effect of harvesting date, immersion treatment and their interaction on the percentage of juice, the percentage of peel, and juice/peel ratio for local sweet lemon fruits.

Harvesting date	The percentage of juice							The average effect of harvesting date
	Immersion treatment							
	T1	T2	T3	T4	T5	T6	T7	
D1	37.91	30.97	36.26	31.55	41.50	34.04	42.62	36.41
D2	38.09	31.17	35.36	31.68	41.68	34.29	42.81	36.44
Average effect of treatments	38.00	31.07	35.81	31.61	41.59	34.16	42.71	D= N.S
L.S.D 0.05	T= 4.131				D×T= 5.842			
Harvesting date	The percentage of peel							The average effect of harvesting date
	T1	T2	T3	T4	T5	T6		
D1	34.34	52.58	48.91	36.52	47.34	49.87	47.53	45.30
D2	41.70	53.74	49.54	40.03	48.69	50.94	49.32	47.71
Average effect of treatments	38.02	53.16	49.23	38.28	48.02	50.41	48.43	D= N.S
L.S.D 0.05	T= 6.209				D×T= 8.781			
Harvesting date	Juice/Peel ratio							The average effect of harvesting date
	T1	T2	T3	T4	T5	T6		
D1	1.174	1.720	1.428	1.268	1.139	1.476	1.127	1.333
D2	1.202	1.746	1.436	1.302	1.166	1.497	1.200	1.364
Average effect of treatments	1.188	1.733	1.432	1.285	1.152	1.486	1.163	D= N.S
L.S.D 0.05	T= 0.2703				D×T= 0.3822			

3- Respiratory speed rate (mg CO_2/Kg/h)

Citrus fruits are considered non-climacteric Fruits, which means that there is no sudden increase in the Respiratory speed rate when the fruit is fully grown, in addition to that the production of ethylene gas in it does not change during ripening and It be at a very small level to the extent that it can only be measured with the use of sophisticated instruments (32). Figure (1) observed that the second date for harvesting the sweet lemon fruits reduced the respiration rate from (5.62 mg CO_2/Kg/hr) on the first date to (5.402 mg CO_2/Kg/hr) in the second date. This trait was significantly affected when treating fruits with Chitosan at a concentration of (20 $g.L^{-1}$) which decreased to (5.423 mg

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CO_2/Kg/hr) compared to untreated fruits, which recorded an increase in the respiratory rate amounted to (5.699 mgCO_2 / Kg/hr). It was also noted that there were significant differences between the interaction values between the harvesting date and the type of solution. The bi-interaction between the first harvesting date and the control treatment (D1T1) gave the highest respiratory rate for sweet lemon fruits amounted to (5.838 mg CO_2 / Kg/hr) compared to the lowest rate for this trait when the fruits of the interaction treatment between the second harvesting date and the treating with calcium chloride solution at a concentration of (1 $g.L^{-1}$) D2T4 which amounted to (5.206 mgCO_2 / Kg/hr) as shown in Table (3). This difference is due to the fact that the second date represents the optimal stage for physiological maturity, which is characterized by slow bio-processes including respiration, which is the preferred stage for storing fruits. As for the role of treating with Chitosan in reducing the respiration rate during storage as shown in Figure (2), It is through the formation of a thin wrapping Film surrounds the fruits, works to reduce gas exchange and prevents the increase in respiratory rate and metabolic activity, thus delaying the ripening and Senescence of the fruits (31, 23, 33).

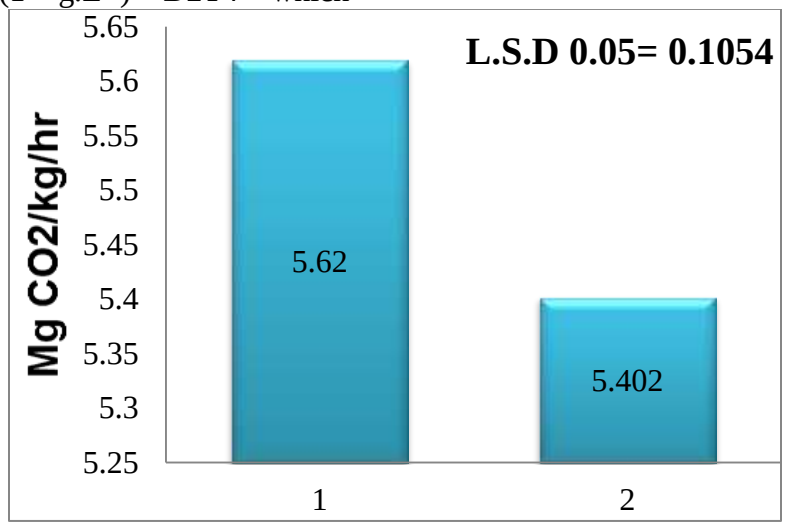

Figure 1: Effect of harvesting date on the respiratory rate for local sweet lemon.

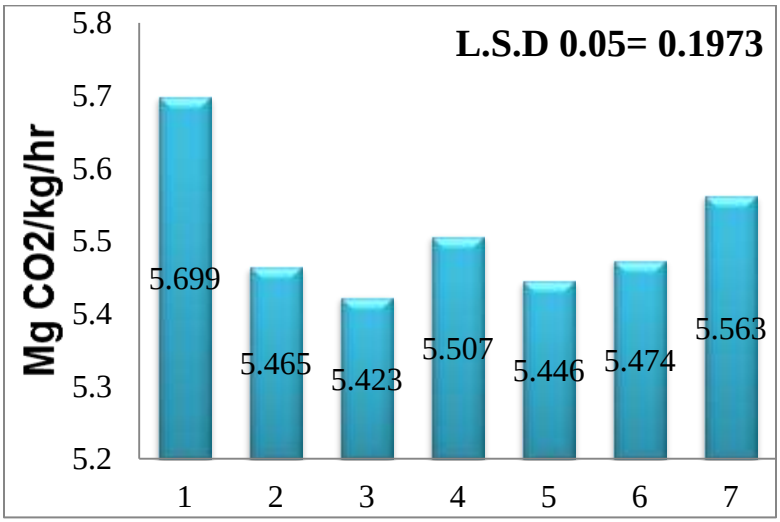

Figure 1: Effect of harvesting date on the respiratory rate for local sweet lemon.

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Table 3: Effect of harvesting date, immersion treatment and their interaction on the respiratory rate for local sweet lemon fruits.

Harvesting date	Immersion treatment						
	T1	T2	T3	T4	T5	T6	T7
D1	5.838	5.770	5.739	5.697	5.636	5.629	5.483
D2	5.561	5.563	5.666	5.206	5.257	5.297	5.642
L.S.D 0.05	D×T= 0.2529						

4- The percentage of damage (%)

Citrus fruits are exposed to many pathogens and physiological causes, starting from the harvesting process and through the transport processes, circulation, and marketing, which negatively affects its quality and storability, and thus damaging it (5). The second harvesting date (D2) has characterized by a significant decrease in the percentage of damage amounted to (7.63%) compared to its rise in the first date (D1) which amounted to (8.35%) as shown in Table (6). As for the effect of the type of solution, the treating with Chitosan solution at a concentration of (10 $g.L^{-1}$) was significantly excelled by giving it the lowest average for this trait amounted to (4.75%) compared to the control treatment, where it recorded the highest average amounted to (11.92%). It is also noted that there were significant differences between the values of bi-interaction, where the interaction between the second harvesting date and the treating with Chitosan solution (10 $g.L^{-1}$) gave the lowest percentage of damage amounted to (4.13%) compared to the control treatment in the first date that gave the highest percentage of damage to the sweet lemon fruit which amounted to (12.58%). Penicillium sp has been diagnosed as the main cause of fruit damage, where it is considered one of the fungi that infect fruits, especially citrus fruits, during storage. It can be noted that the lack of damage is due to the fact that the second date was more appropriate where it represents the optimal stage of physiological maturity, which is characterized by slow bio-processes including respiration, which is the preferred stage for storing fruits. The fruit damage during storage is due to the continuation of bio-processes, including weight loss and the consumption of most of the nutrients stored in the fruits by the respiration process, which led to crossing the ripening stage and entering the Senescence stage, thus reducing its resistance to infection by microorganisms, but the use of Chitosan has contributed to reducing the percentage of damage through urging Chitinase enzyme Which is considered one of the defensive enzymes that stimulate the hydrolysis of Chitin compound in the cell walls of the fungus and then prevents the growth of fungi on the fruits (34, 35).

Table 4: Effect of harvesting date, immersion treatment and their interaction on the percentage of damage for local sweet lemon fruits.

Harvesting date	Immersion treatment							The average effect of harvesting date
	T1	T2	T3	T4	T5	T6	T7	
D1	12.58	5.37	6.87	7.77	7.60	9.64	8.60	8.35
D2	11.27	4.13	5.67	7.70	6.70	9.31	8.63	7.63
Average effect of treatments	11.92	4.75	6.27	7.73	7.15	9.47	8.62	0.659D=
L.S.D 0.05	T= 1.23				D×T= 1.745			

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